A STUDY OF NITROSOTHYMOL AND AMINOTHYMOL WITH PARTICULAR REFERENCE TO SCHIFF'S BASES OF AMINOTHYMOL

By

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INTRODUCTION

It is well known that thymol (2-isopropyl-5-methyl-phenol) because of the alkyl substitutions in its ring has certain pharmacological advantages over other phenols.

Thymol resembles phenol in general activity but its antiseptic efficiency is higher, having a phenol coefficient of 25. The comparative slight solubility of thymol in water reduces its activity as a germicide, but likewise prevents it from being toxic locally. Even after absorption from the alimentary tract, it is only a fourth as toxic as phenol.[1]

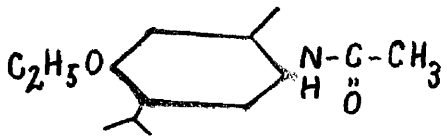
Thymol in a concentration of .02 per cent will prevent the growth of molds while a somewhat higher concentration hinders the action of emulsin. Ascarides in vitro are killed in four hours by a 1-2500 solution of thymol, and owing to the insolubility of this phenol, it is a very eligible agent for the destruction of hookworms and round worms in the bowels.[2]

Foss[3] found that of a number of phenolic sulfur ureides, the one prepared from thymol possessed the greatest germicidal activity toward Bacillus typhosus and Staphylococcus.

Thymol iodide[4] and the more recent chlorthymol[5] are two synthetic derivatives of thymol which have proved effective enough as bactericides and deodorants to warrant official recognition in this country.

Two salols of thymol have been used in medicine but less widely than its halogen derivatives. Pohl[6] introduced thymol carbonate as a safer and more agreeable substitute for thymol when it must be given in large doses for instance against Anchylostomum duodenale. Thymol salicylate[7] has been described in the literature as having antiseptic action.

Jolly[8] recommends thymacetin



(the monoacetyl derivative of p-aminothymolethylether), a compound having the same chemical relationship to thymol that phenacetin

(the monoacetyl derivative of p-aminophenolethylether), has to phenol, as a valuable analgesic and hypnotic when administered in 3 to 15 grain doses.

From the data contained in these citations, it may be assumed that:

1. Thymol is a readily available naturally occurring phenol.

2. Thymol is in many ways a superior phenol from a pharmacodynamic standpoint and its activity may be carried over into a number of its diverse derivatives.

3. If the solubility of thymol in water could be increased without altering its structure too much, it might conceivably function as a more powerful germicide.

4. The deodorant and antiseptic activity of thymol

may be intensified or supplemented by the addition of similarly acting inorganic elements.

5. By substituting certain organic radicals in thymol, particularly in p-aminothymol, it is possible to produce a compound having both analgesic and hypnotic action.

6. Thymol may be rendered safer and more acceptable as an anthelmintic by masking its phenolic group until it reaches the desired site for action.

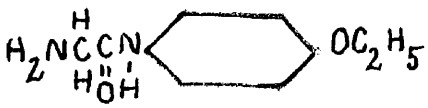
After Cahn and Hepp[9] in 1886 advised the use of acetanilid (monoacetylaniline) as an antipyretic, it was shown that this or any other compound capable of undergoing a partial oxidation in the body to p-aminophenol may be advantageously used to control pain and reduce fever.[10]

Since acetanilid is not entirely free from the poisonous action of aniline, producing dangerous collapse and some destruction of blood cells, certain synthetic compounds patterned after its general formula have been introduced as substitutes for acetanilid. The most important group of these substitutes have been the phenetidin (p-ethoxyaniline) derivatives such as

phenacetin  (acetyl-phenetidin),

(lactyl-phenetidin),

lactophenine  (lactyl-phenetidin),

phenocoll  (glycocoll-phenetidin).

The use of these p-phenetidin derivatives is based on the assumption that after ingestion they are capable of being converted to p-aminophenol. Their degree of safety must necessarily depend on the rate at which this conversion takes place.[11]

Bogert and Connitt[12] have shown that m-cresol is more germicidal than phenol and the toluidines are less poisonous than aniline. This is in agreement with the activity-toxicity data compiled for thymol and moreover indicates that p-aminothymol would be less toxic than p-aminophenol.

The clinical results of hexylresorcinol also indicate that alkyl groups substituted in the ring will enhance the action of the phenolic group and at the same time decrease its toxicity.[13]

From these data it seems not unreasonable to believe that a series of compounds capable of being converted in the body to a p-aminophenol containing one or more alkyl groups substituted directly in the ring might act as analgesics and antipyretics with a decrease in the production of undesired action.

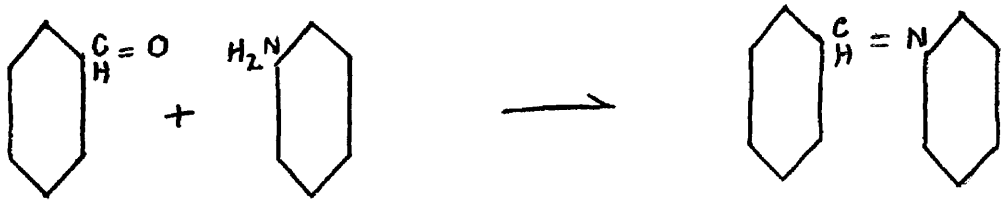
Reasoning from the same data even safer derivatives should result, with the ether of a p-aminophenol which contained alkyl groups substituted directly in the ring.

Schiff's bases or anils are synthetic compounds containing the $R-C(H)=N-R$ grouping formed by the interaction of a primary amine with an aldehyde or ketone whereby the two hydrogens of the amine combine with the oxygen of the car-

bonyl group to split out a molecule of water

$$CH_3 - NH_2 - CH_3C\equiv H^O \text{-----} CH_3N{=}CH - CH_3 - H_2O$$

In 1850 Laurent and Gerhardt[14] condensed benzaldehyde with aniline

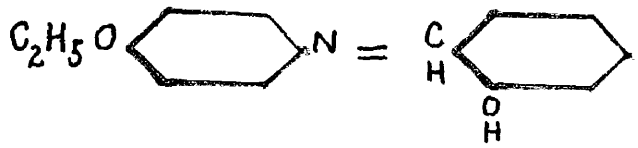


obtaining a product to which they gave the name "Benzoil-anilid". Schiff[15] in 1864 studied this reaction further and condensed aniline with not only aromatic but also with aliphatic aldehydes obtaining compounds possessing the typical anil grouping. Because of his work with compounds of this type they have been identified as Schiff's bases. Since that time Schiff's bases from aromatic amines and aromatic aldehydes have received the most attention from workers in this field.[16]

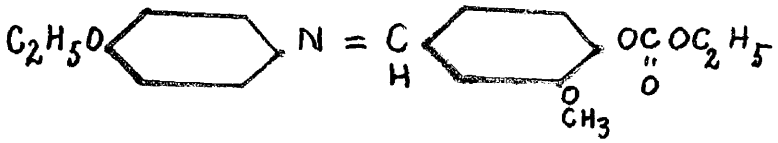
Schiff's bases are generally crystalline compounds which can often be distilled without decomposition. They are weak bases, sparingly soluble in water and form hydrochlorides in non-aqueous solvents but their most characteristic property is the great ease with which they are hydrolyzed by aqueous acids to the amine and the carbonyl compound from which they are derived.[17] They are stable to alkalies.

The possibility of combining pharmacologically active amines and aldehydes to prepare a Schiff's base exhibiting the action of both components has not been entirely overlooked in the past.

Jaquet[18] in 1893 called attention to the fact that "Malakin",



the trade name for Salicylalparaphenetidin, a Schiff's base prepared by condensing p-ethoxyaniline and salicylaldehyde, possessed antirheumatic properties when administered in doses of 8-15 grains. Ottolenghi[19] claims that this compound is slowly absorbed and hence will act on intestinal parasites. "Eupyrin"



"paraphenetidin-vanillinethylcarbonate" is another Schiff's base which was brought forward in 1900 by Overlach[20] who says that it is a superior antipyretic since it produces a gradual but marked fall in temperature which is accompanied by a sense of euphoria rather than a state of depression.

Several Schiff's bases were shown by Glen et. al[21] to be antiseptic in vitro (in serum as well as in aqueous medium) toward ordinary bacteria as Staphylococcus and B. coli and some of them have a therapeutic action on experimental Streptococcus in mice.

The Schiff's base formed from sulfanilamide (p-aminobenzenesulfonamide) with salicylaldehyde, vanillin or resorcyaldehyde, in spite of their slight solubility in water

were found by Raiziss[22] to be more effective than sulfanilamide in saving mice infected with Streptococci. In this study these compounds were administered to the mice "per os" in a mucilage of acacia suspension.

From this previous work two postulations may be made concerning the pharmacological activity of Schiff's bases.

1. A number of Schiff's bases have pharmacological activity. Those possessing activity were made from either a pharmacologically active amine or a pharmacologically active aldehyde.

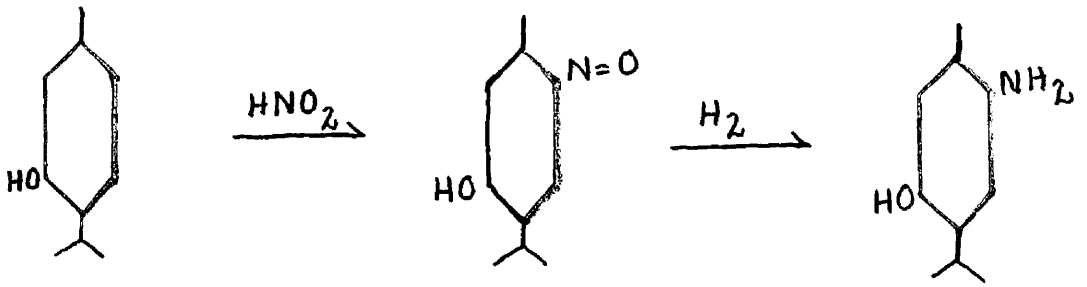
2. The rate of hydrolysis of the R-C=N-R linkage peculiar to the Schiff's bases and the different degrees of solubility of these compounds modify the intensity and the duration of the action of the amines and aldehydes from which they are made.

Thus through Schiff's bases one may approach several chemotherapeutic problems with some expectation of solving them.

The primary object of the studies reported here was to prepare a series of derivatives of p-aminothymol and to examine their pharmacodynamic properties. Among the compounds prepared were several previously known, but which logically belong to the group under investigation. In the preparation of these previously known compounds several hitherto unknown properties were observed and will be described. The pharmacological examination will, it is hoped, shed some light on the effect of chemical constitution on physiological activity, particularly so far as this series

of compounds is concerned.

The aminothymol used in the synthesis of all these compounds was obtained from thymol through nitrosothymol



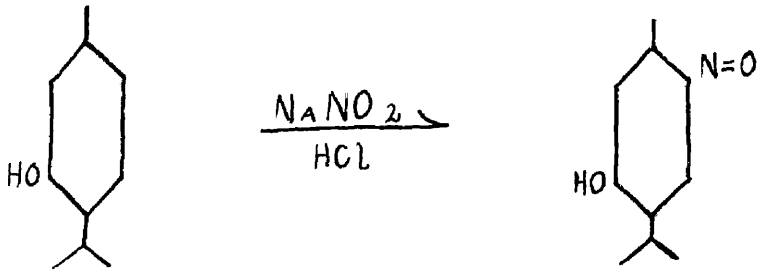
While satisfactory directions for the preparation of these compounds are published, modifications were used, and the results of these will be described.

PART I

p-NITROSOTHYMOL, THYMOQUINONE AND THYMOQUINONEDIOXIME

p-Nitrosothymol has been prepared by several methods[23] but for this work a modification of the method of Kremers and Wakeman[24] was used. One hundred grams (0.66 mole) of thymol was dissolved in 500 ml. of ethyl alcohol and to this was added 500 ml. of concentrated hydrochloric acid. The temperature of this solution was reduced to -5°C. and kept below 0°C. while 72 g. (1 mole) of sodium nitrite was added in divided portions with constant and vigorous stirring.

After the sodium nitrite had been added the reaction mixture became almost solid due to the formation of the insoluble p-nitrosothymol.



The entire mixture was added to 8 l. of water and the p-nitrosothymol was thoroughly washed by careful agitation. It was then filtered out by suction, again washed and finally dried at room temperature.

The p-nitrosothymol was obtained as a buff-colored,

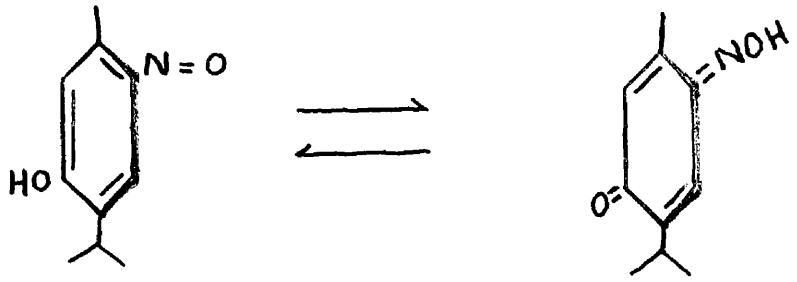
bulky powder which, without further purification, melted under rapid heating at 153-155°C. (uncorr.). It was recrystallized once from benzol to yield practically colorless short needles which melt under fairly rapid heating at 162°C. (uncorr.). Schiff[25] found that p-nitrosothymol, recrystallized from benzol and then precipitated from an alcoholic solution by water, melted at 160-162°C. under rapid heating but melted with decomposition at 155-156°C. when it was slowly heated.

The method outlined here produced practically quantitative yields of p-nitrosothymol of a fair degree of purity.

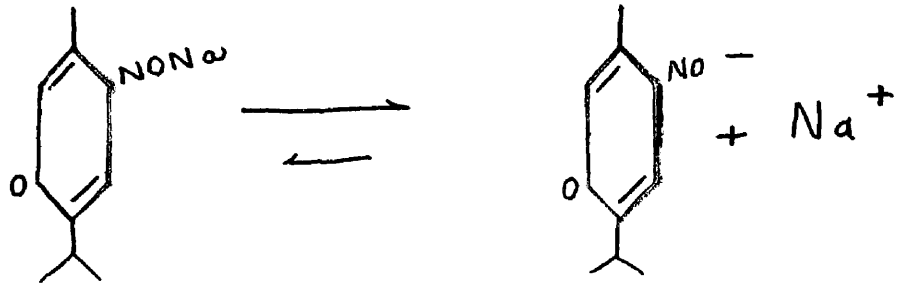
Soon after p-nitrosothymol was prepared its characteristic hydrophobic behavior was observed. This compound was found to repel water so effectively that it was impossible to wet it with aqueous liquids even by prolonged trituration. It is slowly but completely volatile with steam by which method of purification a practically colorless product melting at 158°C. (uncorr.) is obtained.

p-Nitrosothymol is freely soluble in pyridine and in solutions of the hydroxides of alkali metals producing such an intensification of color that even a dilute solution is dark brown. By the careful addition of hydrochloric acid the color of this dark brown solution may be discharged and the light-colored p-nitrosothymol reprecipitated. These color changes may be explained on the basis that the practically colorless p-nitrosothymol, obtained by the action of nitrous acid on thymol, is tautomeric with the more highly

colored thymoquinonemonoxime which is prepared by condensing hydroxylamine with p-thymoquinone and the product in each case may be represented by the tautomeric equilibrium.



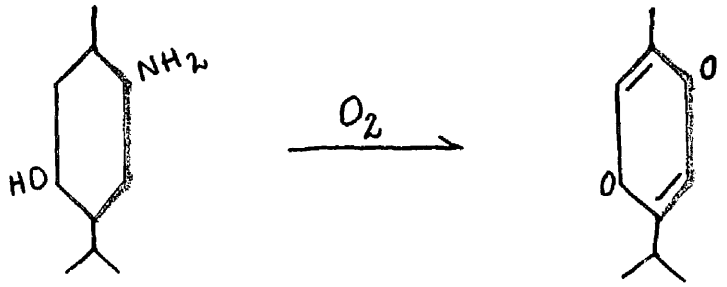
It is generally agreed that the nitroso-benzenoid structure prevails in a neutral medium whereas the oxime-quinonoid form predominates in alkaline and acid solutions.[26] The intense color of the alkaline solution is presumably due to the anions from the highly ionized salt formed from thymoquinonemonoxime and the alkali.[27]



It might be well to add here that either a 20 per cent aqueous solution of sodium carbonate or a 0.15 per cent aqueous solution of calcium hydroxide is capable of effecting this tautomeric transformation to yield in both instances dark brown solutions which behave toward hydrochloric acid in the same manner as did the solution with sodium hydroxide, in that, their color is discharged by the careful addition of hydrochloric acid and at the same time p-nitrosothymol is precipitated in its original state. A 20 per cent aque-

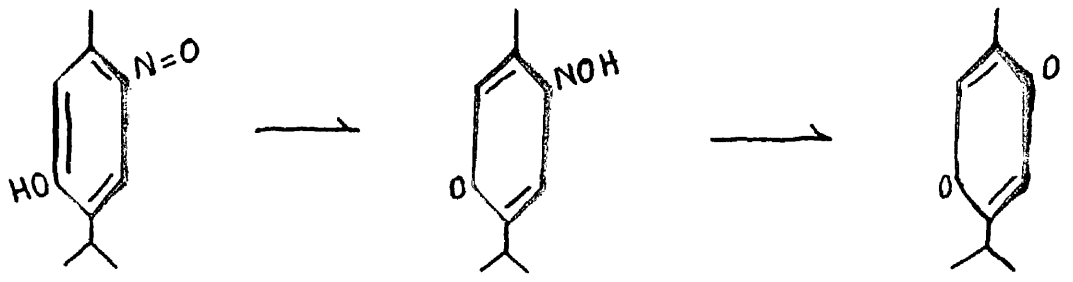
ous solution of potassium bicarbonate was without effect on the tautomeric p-nitrosothymol.

THYMOQUINONE. This particular compound has been prepared by several different methods which have been described in more or less detail in the literature. For example thymoquinone is the principal product resulting when p-aminothymol is oxidized with ferric chloride.[28]



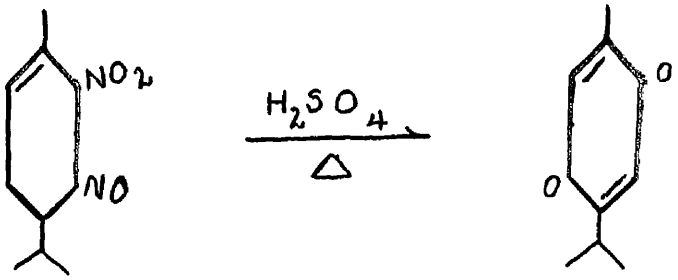
It may also be obtained by oxidizing sulfonated thymol with potassium dichromate[29] or by sulfonating and then oxidizing carvacrol.[30] In spite of the fact that these and other methods have been discovered for preparing thymoquinone it is still quoted at a list price of 55 cents a gram.[31]

Since the readily available p-nitrosothymol is a tautomer of thymoquinonemonoxime, the latter product being obtained by condensing thymoquinone with hydroxylamine, it seemed worthwhile to determine, whether it would be possible to bring about the rearrangement of p-nitrosothymol into its quinonoid-oxime modification and then effect its hydrolysis thereby directly obtaining thymoquinone.



There are several things to indicate that such a reaction should take place. First, an acid medium would tend to convert the tautomeric compound to the quinone structure.[27] Second, the chemical behavior of the quinones indicate that the 6-membered carbon ring has lost its aromaticity and is essentially an unsaturated diketone.[32] Third, the monoximes of ketones may often be hydrolyzed by aqueous acids to yield the original ketone and hydroxylamine.[33]

Up to 1935 there is one reference in the literature to a reaction similar to the one we propose, that used by Wallach and Beschke[34] who obtained thymoquinone (along with other compounds) by warming nitrosites of α-phellandrene with 70% sulfuric acid.



This reaction, however, involves more than a simple hydrolysis of the oxime of a diketone.

Recently Tseng et. al.[35] claim to have obtained an impure thymoquinone in yields ranging from 70 to 77% by gently refluxing p-nitrosothymol for forty-eight hours with 8% hydrochloric acid. They separated the thymoquinone from the reaction mixture by steam distillation and advise that it be entirely freed from the acid as quickly as possible. The results of Tseng et. al. could not be duplicated in our laboratory even though it was modified to the extent of

using various strengths of hydrochloric acid. In no instance were we able to produce enough thymoquinone by this method to warrant the calculation of a yield.

During our experiments it was observed that in practically every case, due to the hydrophobic behavior of p-nitrosothymol, there was little admixture of it with the aqueous hydrochloric acid even after twelve or fifteen hours of refluxing. Throughout the period of refluxing the solid would float on the surface of the liquid and apparently in an almost dry state. Since this lack of contact between the p-nitrosothymol and the medium in which we were attempting its hydrolysis might conceivably hinder the desired reaction, we undertook to bring about an intimate contact between the p-nitrosothymol and hydrochloric acid during the time they were being heated together. This was accomplished by dissolving the p-nitrosothymol in ethyl alcohol and adding concentrated hydrochloric acid to this solution until the desired 7.5% concentration of acid was reached. In another experiment we accomplished the same result by heating the p-nitrosothymol under pressure to 150°C. with 7.5% hydrochloric acid in a sealed glass tube. Neither of these methods yielded thymoquinone and in the latter case there appeared to have been some decomposition of the original substance by the hydrochloric acid.

In a subsequent paper Tseng et. al.[36] state they were able to hydrolyze the monoxime of phenanthraquinone to the extent of 97% by using formaldehyde as a catalyst. Except for the use of formaldehyde, the conditions used in

this experiment for hydrolyzing the oxime were essentially the same as those described in their first paper. We then attempted to hydrolyze the p-nitrosothymol-thymoquinonemonoxime tautomer by refluxing with formaldehyde in 7.5% hydrochloric acid. This method likewise failed to result in the formation of any thymoquinone in spite of the fact that both a 1% and a 10% hydrochloric acid were used in two different experiments.

It seemed reasonable to believe that the carbonyl group in formaldehyde was responsible for the activity which Tseng et. al.[36] have demonstrated that this compound possessed in catalyzing the hydrolysis of phenanthraquinonemonoxime by hydrochloric acid. Too, we had previously shown that it was possible to bring about an intimate mixture of p-nitrosothymol and aqueous acids provided the p-nitrosothymol was first treated with certain organic solvents. Since acetone possesses a carbonyl group and at the same time is capable of facilitating the admixture of aqueous liquids and p-nitrosothymol it was chosen as a catalyst in the next experiment.

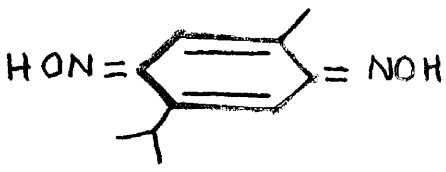
Three and a half grams (0.02 mole) of p-nitrosothymol was triturated with 2.5 ml. (0.04 mole) of acetone to form a paste. To this was added 50 ml. of 7% hydrochloric acid and then the entire mixture was transferred to a small boiling flask after which it was subjected to twenty-four hours of refluxing. The mixture was refluxed by the use of a direct flame the heat of which was controlled by means of an asbestos gauze. After about ten hours of refluxing

the appearance of an appreciable quantity of yellow oil around the cooler portion of the boiling flask indicated that a fair amount of thymoquinonemonoxime had been hydrolyzed into thymoquinone. After the reaction mixture was refluxed for another ten hour period it was distilled with steam. By this means 1.2 g. of light orange-colored, plate-shaped crystals were obtained. These crystals possessed the characteristic odor of thymoquinone and without further purification melted at 45°C. (corr.). The melting point of thymoquinone is given in the literature as 45.5°C.[37] A yield of 1.2 g. of thymoquinone from 3.58 g. of thymoquinonemonoxime represented 36% of the theoretical.

In another experiment 3.58 g. (0.02 mole) of p-nitrosothymol was dissolved in an excess of acetone and then to this was added concentrated hydrochloric acid alternately with more acetone until a perfect solution resulted, the strength of which was 7% with respect to hydrochloric acid. This solution was gently refluxed for ten hours but the formation of thymoquinone could not be detected in this instance.

An attempt to hydrolyze thymoquinonemonoxime in an alkaline solution was likewise fruitless.

THYMOQUINONEDIOXIME. In view of the similarity in structure which thymoquinonedioxime



shows to the several compounds just described it was deemed fitting to conclude the immediate series of experiments by preparing the dioxime of thymoquinone and comparing its chemical behavior with that of the compounds just studied.

The preparation of thymoquinonedioxime was accomplished by the following method. There was dissolved 5.37 g. (0.03 mole) of crude p-nitrosothymol in 35 ml. of ethyl alcohol and to this solution was added 2.07 g. (0.03 mole) of hydroxylamine hydrochloride. The mixture was then refluxed on a water bath until a light yellow solid began appearing in the liquid. After this another equivalent (0.03 mole) of hydroxylamine hydrochloride was added and the refluxing continued for two hours. The mixture was allowed to cool and the yellowish-white crystalline precipitate was collected by filtration and placed in a desiccator. After the crystalline material had thoroughly dried it appeared to be comparatively pure and when weighed was found to be 4.5 g. which represented a yield approximating 70% of the theoretical. The compound was then recrystallized from ethyl alcohol until practically colorless microscopic crystals were obtained which on heating in a melting point apparatus darkened at 225°C. (uncorr.) and decomposed at 235°C. (uncorr.). Kehrmann and Messinger[38] found that when recrystallized thymoquinonedioxime was subjected to a melting point determination it darkened at a temperature of over 200°C. and decomposed at 235°C.

Thymoquinonedioxime being definitely of the oxime-quinonoid structure and unable to shift to the tautomeric

nitroso-benzenoid modification should be theoretically even more easily hydrolyzed than the thymoquinonemonoxime since the latter compound is capable of rearranging to the p-nitrosothymol tautomer and as such could not possibly yield thymoquinone by undergoing hydrolysis.

The following experiment was conducted to determine whether thymoquinonedioxime will undergo hydrolysis under the conditions generally used for hydrolyzing dioximes of diketones.

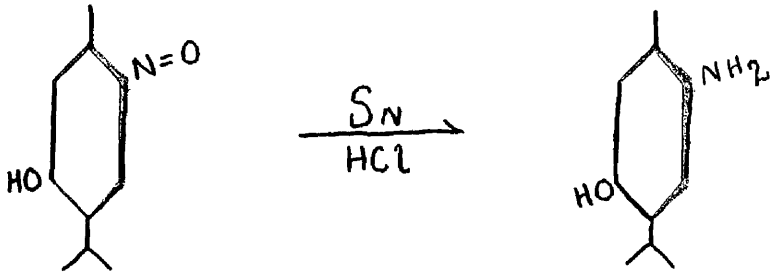
Four grams (approximately 0.02 mole) of thymoquinonedioxime was refluxed with 50 ml. of water for thirty minutes but there were no indications of hydrolysis taking place. The mixture was then allowed to cool and to it was added 2.5 ml. of concentrated hydrochloric acid after which it was again refluxed. Very soon after boiling had commenced, following the addition of the acid, the formation of some thymoquinone was indicated by the appearance of yellow crystals in the upright condenser tube. The formation of thymoquinone ceased soon after it had begun and continued refluxing did not increase the yield. It was interesting to note that along with the small amount of thymoquinone formed there was also found some thymoquinonemonoxime. (This suggests that one of the oximino groups is removed more rapidly than the other.) The entire mixture was then subjected to steam distillation but only negligible amounts of thymoquinone or thymoquinonemonoxime were isolated which denoted that very little hydrolysis had taken place.

PART II

p-AMINOTHYMOL AND THYMOHYDROQUINONE

In this study we were interested in preparing a quantity of p-aminothymol for two reasons. First, p-aminothymol is one of the best known derivatives of p-nitrosothymol and hence its preparation could not be ignored in a study of p-nitrosothymol and its derivatives. Second, it was necessary to expedite as far as possible the production of p-aminothymol since it was to serve as the starting material for a number of Schiff's bases, a study of which constitutes a portion of this problem.

A search of the literature revealed that p-aminothymol results when p-nitrosothymol is reduced with tin and hydrochloric acid.[39]



The reduction of p-nitrosothymol to p-aminothymol is likewise conveniently carried out by the use of hydrogen sulfide in the presence of ammonium hydroxide.[23] It has also been prepared by treating p-nitrosothymol in warm benzol with phenylhydrazine[40] and by reducing carvoxime in sulfuric acid with sulfur dioxide.[41]

Of these four methods we employed the one involving the use of hydrogen sulfide as the reducing agent since it has been shown further[24] that this method produces a fair yield of p-aminothymol and moreover it is relatively easy to separate the desired product from the reaction mixture. The details of the ammonium sulfide reduction method which we used are as follows.

Ninety grams (0.5 mole) of crude dry nitrosothymol was dissolved in 3 l. of 10% ammonia water yielding a dark brown solution which was separated from a small amount of impurities by filtration. This ammoniacal solution was placed in a 5 l. Erlenmeyer flask fitted with a two-hole rubber stopper and was treated with a stream of hydrogen sulfide gas which was generated and passed into the solution in this manner. A Kipp generator, charged with ferrous sulfide and diluted hydrochloric acid, was tightly connected to a water-filled wash bottle which in turn was connected to the 5 l. Erlenmeyer flask by means of an L-shaped tube the long arm of which extended well down into the ammoniacal solution of p-nitrosothymol. Through the other hole of the two-hole rubber stopper in the 5 l. Erlenmeyer flask was passed a short piece of glass tubing over the exposed end of which was slipped a short piece of thick-walled rubber tubing fitted with a screw clamp. The air was removed from the system by opening the exit from the Erlenmeyer flask and then starting the generation of hydrogen sulfide in the Kipp generator. As soon as the odor of hydrogen sulfide was perceptible in the room the exit from the 5 l.

Erlenmeyer flask was tightly closed by means of the screw clamp and the reaction allowed to continue. After one or two hours the solution had lost much of its color and a white precipitate had begun to form. Soon the entire mixture was almost solid due to the formation of p-aminothymol. After it was observed that the reaction mixture had ceased to take up hydrogen sulfide the generation of the gas was stopped and the p-aminothymol filtered out by suction. It was preserved against oxidation according to the direction of Kremers and Wakeman[24] by quickly converting it to the hydrochloride salt. This was done by transferring the p-aminothymol from the Buchner funnel to 800 ml. of 4% hydrochloric acid from which approximately 100 g. of p-aminothymol hydrochloride could be separated as needle-shaped crystals. This yield represented 75% of the theoretical amount. For approximately the same amount of p-aminothymol Kremers and Wakeman[24] used 1600 ml. of a 2% solution of hydrochloric acid and then evaporated the mother liquor to obtain a second crop of crystals.

We found several advantages in using the Kipp generator in a closed system for reducing p-nitrosothymol to p-aminothymol. First, the reduction can be carried out in the laboratory without the danger of breathing hydrogen sulfide nor the inconvenience of having to use a hood. Second, the reduction can be started at any time and after the reaction is completed the product can be worked up when convenient. Third, it is possible that the pressure produced by the Kipp generator might speed up the reaction and at any

rate the liquid containing the freshly reduced p-aminothymol was kept saturated with hydrogen sulfide which protected the p-aminothymol from oxidation while it was being filtered out.

To determine whether p-nitrosothymol could be reduced to p-aminothymol by hydrogen sulfide in the absence of ammonia, the following brief experiment was conducted.

Nine grams (0.05 mole) of p-nitrosothymol was dissolved in 150 ml. of ethyl alcohol and treated with hydrogen sulfide in the apparatus just described. The alcoholic solution absorbed a considerable amount of hydrogen sulfide to give a heavy yellow precipitate which was later shown to be sulfur. The solution was exposed overnight to the hydrogen sulfide under pressure from the Kipp generator. The sulfur was filtered out and the filtrate quickly acidified by the addition of 6 ml. of concentrated hydrochloric acid. By evaporating the alcohol from this solution 3 g. of solid material was obtained which was recrystallized from diluted hydrochloric acid and shown to be p-aminothymol by a mixed melting point determination. A yield of 3 g. represented approximately 30% of the theoretical.

The possibility of reducing p-nitrosothymol to p-aminothymol by hydrogenation with a catalyst next suggested itself to us. On reviewing the literature it was found that Cusmano[42] is the only worker to have reported an attempt to use this method of reduction for converting p-nitrosothymol to the corresponding amino compound. He found that by shaking an ether or acetone solution of p-nitrosothymol with

platinum black in an atmosphere of hydrogen about half of the theoretical amount of p-aminothymol was obtained.

To study this reaction further and to determine if it were not possible to more effectively reduce p-nitrosothymol to p-aminothymol by catalytic hydrogenation the following procedures were carried out.

The catalyst used for the hydrogenation was finely divided palladium deposited on charcoal according to the method of Ott and Schroter.[43] Two grams of animal charcoal were shaken with 0.5 g. of palladous chloride in 100 ml. of water in an atmosphere of hydrogen until no more hydrogen was absorbed. The catalyst was filtered off, washed with absolute ethyl alcohol and then preserved in an evacuated desiccator until it was needed.

Eighteen grams (0.1 mole) of p-nitrosothymol was dissolved in 350 ml. of absolute ethyl alcohol. The solution, along with the palladium catalyst, was placed in a 500 ml. round-bottom flask which was attached by means of a ground glass connection to a hydrogen delivery train. Except for one short rubber connection leading from the cylinder of hydrogen this apparatus was made entirely of glass and was assembled according to the directions of Hartung[44] who successfully used it in reducing a series of oximes and nitriles. The reduction was carried out in an atmosphere of hydrogen which was provided for the reaction by evacuating the apparatus three times and introducing hydrogen after each evacuation. The hydrogen was led through a large graduated cylinder and supplied throughout the reac-

tion at a pressure of approximately 20 inches of water. Agitation of the flask was begun and within forty minutes the required 4,500 ml. (0.2 mole) of hydrogen had been absorbed with a considerable evolution of heat. The solution was then filtered to remove the catalyst but the difficulty encountered in completely removing the finely divided charcoal prolonged this operation beyond the time usually required for a simple filtration. The first few drops of the solution which were obtained free of charcoal were decidedly violet-colored and before the filtration was completed the color of the solution had become so dark that it was almost black. This denoted considerable oxidation of the p-aminothymol which we attempted to check by the addition of a sufficient amount of hydrochloric acid to form the corresponding salt of the free amine. Evaporation of the alcohol yielded a black solid from which no p-aminothymol hydrochloride could be obtained by the usual method of recrystallization from boiling diluted hydrochloric acid. The failure to obtain p-aminothymol came as a surprise in view of the fact that during the reaction the calculated 0.2 mole of hydrogen was absorbed. Busch and Schulz[45] using the same catalyst deposited on calcium carbonate found that nitrosobenzene was reduced by hydrogen to azobenzene instead of aniline as would be expected. As a possible explanation of this they suggest that some of the nitrosobenzene was reduced to aniline which in turn condensed with unchanged nitrosobenzene to produce azobenzene

This appears an unlikely explanation for our difficulty since the calculated 0.2 mole of hydrogen was absorbed during the reaction and moreover it would be expected that hydrogenation with a palladium catalyst would reduce a nitroso compound further than to the corresponding azo derivative.[46]

Schwab[47] found that the oxidation of a mixture of α-naphthol and p-phenylenediamine to an indophenol was catalyzed by the presence of charcoal. Data are available which indicate that indophenols are responsible for colors produced when p-aminophenols are oxidized.[51] In view of these facts it is possible that the presence of charcoal in our reaction mixture caused the very rapid oxidation of the p-aminothymol which took place after the alcoholic solution of this compound was exposed to the air.

It was then decided to reduce the p-nitrosothymol in the presence of hydrogen chloride which would forestall oxidation of the p-aminothymol by converting it immediately to the hydrochloride.

To accomplish this, 18 g. (0.1 mole) of p-nitrosothymol was dissolved in 280 g. of absolute ethyl alcohol contained in a tared vessel. Dry hydrogen chloride was passed into the solution until it had absorbed 4 g., a quantity representing a 10% excess of the calculated amount. The reduction was carried out in the exact manner described in the previous reduction with palladinized charcoal. This reaction did not proceed as rapidly as the first one nor was there as much heat evolved even though the calculated quantity of 4,500 ml. (0.2 mole) of hydrogen was absorbed

within four hours. The solution was filtered by gravity to remove the catalyst and after the alcohol had been evaporated on a water bath the crude p-aminothymol hydrochloride was obtained. The product was colored but after washing it by suction with several small portions of ethyl acetate a powder resulted which was almost white. After completely drying the powder it weighed 19 g. which represented a yield of 94.5% of the theoretical. It melted at 252°C. (uncorr.). The literature[48] records the melting point of p-aminothymol hydrochloride as 255°C. If the practically colorless p-aminothymol hydrochloride was dissolved in ethyl alcohol and the alcohol allowed to evaporate spontaneously, extremely fine needle-shaped white crystals were obtained.

The satisfactory yield which had been obtained with the palladium catalyst led us to investigate the efficiency of the Adams platinum catalyst[49] in bringing about this same reduction. The catalyst is actually platinum black which is prepared by reducing platinic oxide in the presence of hydrogen. A description of the apparatus and an outline of the procedure in which the Adams catalyst was used will be included here since the method in general differed from the one just described.

One gram of platinic oxide was placed in a 500 ml. Kjeldahl flask and to this was added 18 g. (0.1 mole) of p-nitrosothymol previously dissolved in 350 ml. of ethyl alcohol. The Kjelhahl flask was securely stoppered by means of a two-hole rubber stopper through each hole of which was passed a short piece of glass tube bent at a right angle.

To one of these tubes was attached a piece of pressure tubing which connected the Kjeldahl flask to a source of hydrogen the pressure of which could be determined by a gauge and controlled with a check valve. The hydrogen pressure in the Kjeldahl flask was further controlled by connecting it by means of the other short glass tube in the two-hole rubber stopper in the Kjeldahl flask to a column of mercury. The flask was wedged in the wooden cradle of a mechanical agitator and after hydrogen was admitted under a pressure of 10 pounds the flask was agitated. Approximately 5 l. of hydrogen was absorbed within four hours after agitation of the flask was begun. The calculated quantity of hydrogen to reduce the weight of p-nitrosothymol used was 4.5 liters or 0.2 mole, but the additional 0.5 liters could well have been used in reducing the platinic oxide to platinum black. After the gauge indicated that no more hydrogen was being absorbed the valve on the hydrogen tank was closed and the flask carefully opened. The solution was immediately treated with 4 g. (0.11 mole) of hydrogen chloride previously dissolved in 50 ml. of ethyl alcohol. The platinum black was filtered off by suction and the filtrate evaporated almost to dryness on a water bath. This yielded a dark gray powder which was then further dried over calcium chloride in vacuo. This powder could be obtained as almost white needle-shaped crystals by washing it on a Buchner funnel with several small portions of ethyl acetate. The appearance of these crystals indicated that they were p-aminothymol hydrochloride and this was confirmed by determining that their mixed melting point

with a pure sample of this compound was 254°C. (uncorr.).

Analyses: Calculated for $C_{10}H_{16}ONCl$ Cl = 17.60

Found Cl = 17.90

The quantitative determination for chlorine was made by the well known Parr bomb method.

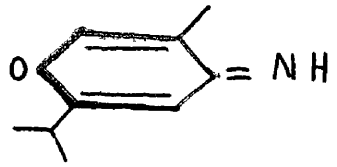
It is well known that p-aminophenols are easily oxidized to purple-colored compounds, some of which are of undetermined complexity. Lossen[50] has suggested that the marked coloration which p-aminophenols undergo on oxidation be used as a means of identifying this class of compounds. p-Aminothymol is likewise so unstable toward oxidation that it can be kept only in the form of one of its salts, otherwise, the free amino compound is quickly converted to a violet-colored dye.

By a qualitative experiment it was found that the violet-colored compound could be obtained by passing air for forty-eight hours through a 10% alcoholic solution of p-aminothymol. The dye was also prepared by shaking for four hours 1 part of p-aminothymol hydrochloride dissolved in 20 parts of ethyl alcohol with 0.5 of a part of mercuric oxide or lead dioxide or manganese dioxide. In each case the resulting product was a black solid melting between 85° and 90°C. (uncorr.). It dissolved in ethyl alcohol to give a deep purple solution which was distinctly colored at a dilution of 1 to 100,000. The color of a 0.1% alcoholic solution of the dye was discharged by an excess of hydrogen sulfide, sodium bisulfite or formaldehyde. It was shown that if the solution which had been decolorized by hydrogen sulfide was exposed

to the air, the violet color reappeared in a short time. The color of the dye was affected by the addition of an acid or a base. In basic solution its color was distinctly green while the addition of an acid changed it to a purplish red.

Gnehm and Bots[51] have shown that an indophenol $HN{=}C_6H_3(CH_3){=}N{-}C_6H_4OH$ tautomeric with $H_2N{-}C_6H_3(CH_3){-}N{=}C_6H_4O$ is formed when o-toluidin and p-aminophenol are oxidized by ferric chloride in the presence of sodium acetate. They also showed that this indophenol was capable of being reduced by sodium sulfide to a colorless compound. Another worker[52] has shown that the color of the indophenol prepared by Gnehm and Bots[51] has a different color in acid and alkaline solution.

In view of these data and assuming that the first product formed by oxidation of p-aminothymol is this quinone-imid



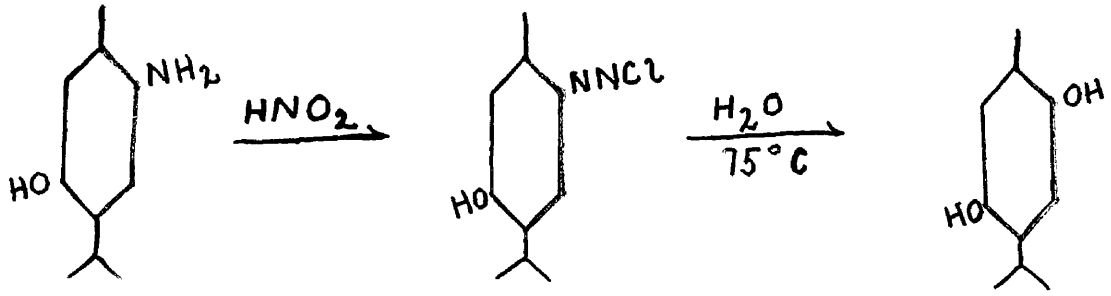
it is possible that the ultimate oxidation product of p-amino-thymol is an anil formed in this fashion

Whether this formula represents the entire molecule or just one of the units from which a more complex molecule is composed was not determined. However, it could undergo the same sort of tautomerism which Gnehm and Bots[51] illustrate for the indophenol which they prepared by oxidizing a p-aminophenol. Furthermore the oxidation of p-aminophenol to the violet-colored dye took place most readily in absolute alcohol which is an ideal medium for anil formation. The reaction was apparently catalyzed by charcoal and it has been shown[47] that this catalyst likewise aids in the formation of certain anils. A further relation that the violet-colored dye showed to indophenols was its definite decoloration by ascorbic acid.[53]

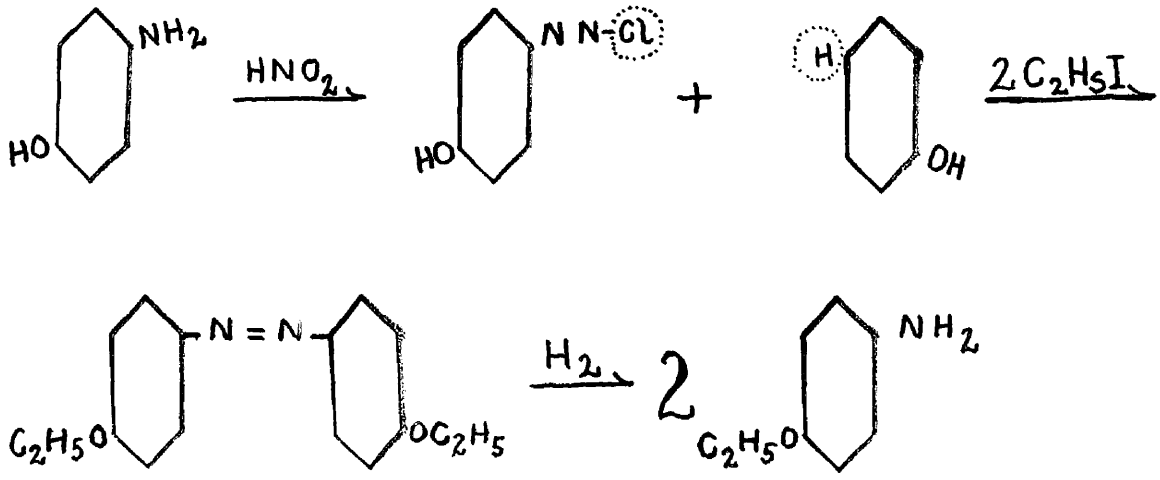
The tendency of p-aminothymol to oxidize to the highly colored compound just described interferred considerably with the purification of even the hydrochloride of this compound. Kremers and Wakeman[24] stated they were able to obtain p-aminothymol hydrochloride in colorless needles by recrystallizing it from highly diluted hydrochloric acid with the use of a little decolorizing carbon. Tolstoouhov[54] has patented a process for purifying p-aminothymol which consists of dissolving the compound in a dilute acid, extracting the impurities with ether, and then reprecipitating the p-aminothymol by alkalinizing the solution with sodium sulfite. We were more successful in obtaining colorless crystals of p-aminothymol hydrochloride by recrystallizing it from diluted hydrochloric acid which contained in addition to the decolorizing charcoal several zinc shot for each

liter of solvent.

THYMOHYDROQUINONE. An attempt to diazotize p-aminothymol seemed worth while for two reasons. First, it would be a step toward converting p-aminothymol to thymohydroquinone



Second, the diazonium salt would constitute an intermediate from which it might be possible to prepare medicinally important derivatives of thymol. For example the method of Riedel[55] for preparing the ethyl ether of p-aminophenol might be used to synthesize the ethyl ether of p-aminothymol. Riedel's method calls for diazotizing p-aminophenol, coupling the product with phenol, ethylating, and then reducing this compound to obtain two molecules of p-aminophenolethylether



If thymol and p-aminothymol were substituted for phenol and p-aminophenol respectively in the reaction indicated above, two molecules of p-aminothymolethylether should result.

Our first efforts to obtain thymohydroquinone by diazotizing p-aminothymol and then treating the diazonium salt with water heated to 70-100°C. were not successful. Since the failures resulted from one general procedure only one unsuccessful run will be described.

Sixty-six grams (0.25 mole) of p-aminothymol sulfate was dissolved in 2 l. of water containing 55 ml. of concentrated sulfuric acid. The solution was cooled to 0°C. and then treated under constant stirring with 18 g. (0.24 mole) of sodium nitrite in divided portions. The solution was allowed to stand overnight after which time it was of a light orange color and contained a small amount of a flocculent precipitate. The entire mixture was heated on a steam bath for four hours at a temperature of 65-75°C. This resulted in the formation of a considerable quantity of a red gum which possessed the characteristic sharp odor of a quinone. The mixture was then cooled to 0°C. to give a small crop of yellowish needles which were shown to be thymohydroquinone by a melting point determination. The best yield of thymohydroquinone obtained by this procedure was 6% of the theoretical.

It was presumed that the formation of the red gum was due to a reaction between thymoquinone and thymohydroquinone both of which were present in the reacting mixture. This is tenable in view of the well known reaction between

quinone and hydroquinone to form the more highly colored quinhydrone. It was believed that if the thymohydroquinone could be removed from the reaction mixture as quickly as it was produced by the diazonium salt the formation of the red gum would be reduced to a minimum and the yield of the thymohydroquinone correspondingly increased. To accomplish this we attempted to use a method devised by Rinckes[56] which consisted of dropping the cooled diazonium solution on a mixture of one volume of sulfuric acid and one volume of water through which a strong stream of steam was being blown. The application of this method depends on the phenol being distilled with the steam and hence proved worthless for our purpose since we found, contrary to expectation, that thymohydroquinone was not volatilized by steam.

Zeigler et al.[57] have shown that the chance of intermolecular reaction is considerably diminished if the solution of the reacting substances be made sufficiently dilute. We thought that the application of this principle might reduce the amount of red gum formed when the diazonium compound was heated in water for several hours in an attempt to convert it to the phenol. The following experiment was conducted to determine this. The directions followed in producing the diazonium compound were taken from Gattermann's "Laboratory Methods of Organic Chemistry"[58].

Two grams (0.01 mole) of p-aminothymol was added to 50 ml. of water containing 4 ml. of concentrated hydrochloric acid. The mixture was cooled to 2°C. and to it was slowly added under constant stirring 0.69 g. (0.01 mole) of sodium

nitrite previously dissolved in 10 ml. of water. As the nitrite solution was added the undissolved p-aminothymol hydrochloride disappeared and a brown foam began to form on the surface of the solution. Soon the foam became a dark colored flocculent precipitate which was later removed by filtration and found to weigh after drying 0.5 g. After removing the brown precipitate the green solution of the diazonium salt was poured into 2 l. of strongly agitated boiling water. This was accomplished without the formation of the red gum and very little appeared to be formed during the remainder of the experiment. The solution was allowed to cool to room temperature and then evaporated under reduced pressure to about 1/20 of its original volume. This greatly reduced volume was cooled to 0°C. whereupon a crop of short needle-shaped yellowish crystals precipitated. These crystals possessed a shape typical of thymohydroquinone and their melting point of 138°C. (uncorr.) indicated further that they were thymohydroquinone. Carstangen[59] gives the melting point of thymohydroquinone as 139.5°C. This reaction was attempted only the one time and the 0.7 g. of thymohydroquinone obtained in the above experiment represented a yield of 43% of the theoretical.

PART III

SCHIFF'S BASES WITH p-AMINOTHYMOL

In setting out to prepare a series of Schiff's bases from aldehydes or ketones and the primary aromatic amine, p-aminothymol, the choice of the carbonyl compound was important for these three reasons.

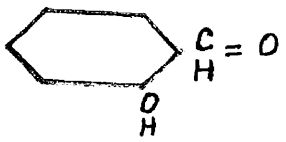
1. It has been shown that a Schiff's base prepared from an aromatic amine and a lower aliphatic aldehyde is very likely to polymerize[60] and hence it would be difficult to isolate the desired compound as an entity.

2. Under certain conditions[61] an aromatic amine condenses with a simple aliphatic ketone to yield a regular Schiff's base while under other conditions[62] these compounds react to give much more complex products, mainly substituted quinolines. These are beyond the scope of the present study.

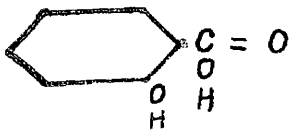
3. The conditions for preparing Schiff's bases from aromatic amines and aromatic ketones are quite different and more severe than those necessary to condense aromatic amines with other carbonyl compounds.[63] For this reason the aromatic ketones were not used in this study but will be reserved for a future investigation.

In view of the foregoing facts, it seemed that of the classes of compounds containing the carbonyl group, the aromatic ~~amines~~ aldehydes were best suited for the synthesis of a series of Schiff's bases with the compound being studied,

namely p-aminothymol. Too, it was recognized that certain aromatic aldehydes possess potential properties in common with, and augmentative to those of p-aminophenols. For example salicylaldehyde (o-hydroxybenzaldehyde)



is readily oxidized to salicylic acid (o-hydroxybenzoic acid)



a compound whose derivatives are widely used for their analgesic and antipyretic activity.

A good many observations have been made concerning the general reaction between aromatic amines and aromatic aldehydes in preparing Schiff's bases. Other workers in developing methods to bring about this condensation have been enabled to point out difficulties which are likely to be encountered in attempting the reaction.

Ordinarily a simple aromatic amine and a simple aromatic aldehyde condense smoothly to yield the corresponding Schiff's base, but such is not always the case when the amine and/or the aldehyde contain groups substituted in the ring. For example several workers have observed that steric hindrance decreases, and in some instances removes, the tendency of aromatic amines to condense with aromatic aldehydes to form Schiff's bases. Lowy and Downey[64] found that 2,4,6-tribromoaniline would not condense with 2,4-dinitro-

benzaldehyde after eight hours of heating in 95% ethyl alcohol but did condense after two hours of heating in glacial acetic acid. Hann et al.[65] pointed out that 5-chlorovanillin would not react with p-nitroaniline; 2,4-dichloroaniline nor trinitroaniline to produce a Schiff's base. The position of the nitro groups with respect to the amino group in dinitrotoluidin was found by McGookin[66] to influence the capacity of this compound to form Schiff's bases.

It is most significant, especially in so far as this study is concerned, that the ease of hydrolysis of a Schiff's base is affected by the substituent groups in the compounds from which the Schiff's base was prepared. Reddelien[67] states that the hydrolysis of Schiff's bases by acids is influenced by the groups substituted in the compound. Langman et al.[68] found that a nitro group, especially an ortho-nitro group, on the aldehyde portion of a Schiff's base reduced its ability to be hydrolyzed.

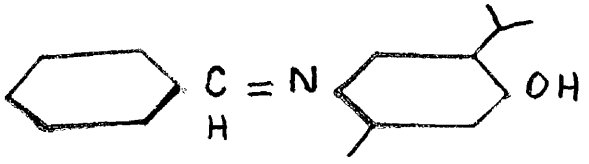
To form Schiff's bases from amines and aldehydes reluctant to condense, and in some instances to increase yields, various condensing agents have been brought forward. Since the elimination of water is necessary for the reaction to proceed, dehydrating agents, among other things, have been used both with and without heat to bring about the condensation. Foster and Thornley[69] used anhydrous sodium sulfate to condense aniline with camphorquinone. Odell and Hines[70] suggested the use of potassium pyrosulfate as a dehydrating agent in condensing aniline and benzaldehyde. Pyl[71] was able to form certain Schiff's bases by condensing

the proper amine and aldehyde in diluted alcohol saturated with carbon dioxide.

Where the amine intended to be condensed into a Schiff's base is previously salified, as all p-aminophenols must be to prevent them from oxidizing, it has been found advantageous in certain cases to treat the amine salt with a weak alkali just prior to the reaction. Sodium bicarbonate[72] and sodium acetate[73] have been used for this purpose.

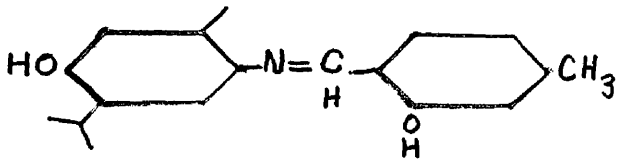
We found that p-aminothymol, even though it contained three groups substituted in its ring and was available only as the hydrochloride salt, could be condensed with aromatic aldehydes to form Schiff's bases. However, we were compelled to use almost as many methods as we obtained compounds.

As far as could be ascertained only three Schiff's bases from p-aminothymol had previously been prepared. Plancher[74] was able to prepare benzylidene-p-aminothymol



by heating freshly distilled benzaldehyde and p-aminothymol for two hours at a temperature of 120-150°C. In a similar manner he condensed cuminaldehyde (p-isopropylbenzaldehyde) and m-nitrobenzaldehyde with p-aminothymol to obtain the corresponding Schiff's base. Plancher did not report his yields, but did record various properties; e.g., the respective melting points are: benzaldehyde 148°C., cuminaldehyde 153°C., and m-nitrobenzaldehyde 161°C.

2-HYDROXY-4-METHYLBENZYLIDENE-4-AMINOTHYMOL.



The p-homosalicylaldehyde used to prepare this compound was obtained from the Eastman Kodak Company and melted at 53-54°C.

p-Homosalicylaldehyde 1.36 g. (0.01 mole) was dissolved in 40 ml. of 50% ethyl alcohol by first dissolving the aldehyde in 20 ml. of 95% ethyl alcohol and then adding 18 ml. of water. To this solution under constant stirring was added in divided portions 2 g. (0.01 mole) of p-aminothymol hydrochloride. During the addition of the p-aminothymol hydrochloride a yellow precipitate began to appear. Stirring was continued for thirty minutes after the last portion of the amine was added and twenty-four hours later there was filtered from the reaction mixture a yellow crystalline solid which after drying weighed 3 grams. The filtrate was distinctly acid to litmus. The yellow solid was kept in a desiccator over calcium chloride for several days during which time it darkened in color and finally became a deep lemon yellow in which was visible some dark orange-colored spots.

This change in color was expected since it is well known[75] that many Schiff's bases, especially those prepared with aromatic aldehydes containing an ortho-hydroxyl group, do undergo color changes. The color changes are thought to be due to the formation of tautomeric modifications which are usually spoken of as the red and yellow forms. The forces which bring these changes about have not been definitely

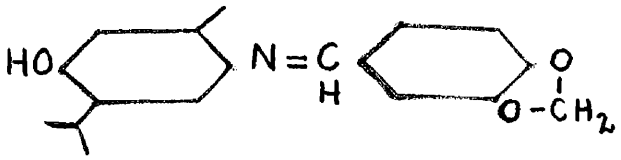
agreed upon[76] and will not be further dealt with here since this will be the subject of a future investigation.

Each time the yellow modification was recrystallized from 40% ethyl alcohol a larger portion of it was converted to the orange-colored form which was less soluble in the recrystallizing solvent than the yellow modification. After the third recrystallization the compound was recovered solely as its orange-colored modification in very fine needle-shaped crystals which melted at 154°C. (corr.).

A sample of the orange-colored compound after being dried for one hour at 110°C. retained its color and lost no weight which indicated that it contained neither alcohol nor water of crystallization. It melted to a red oil at 155°C. (corr.). This Schiff's base was prepared twice by the method outlined above with an average yield of 85% of the theoretical.

Analyses:	Calculated for $C_{18}H_{21}O_2N$	N = 4.90
	Found	N = 4.88

3,4-METHYLENEOXYBENZYLIDENE-4-AMINOTHYMOL. Heliotropin



or Piperonal (3,4-methyleneoxybenzaldehyde) the aromatic aldehyde from which the above Schiff's base was prepared has been used in medicine to a limited extent as an antipyretic and an antiseptic.[77]

Three grams (0.02 mole) of piperonal was dissolved in 12 ml. of dehydrated ethyl alcohol to give an almost saturated

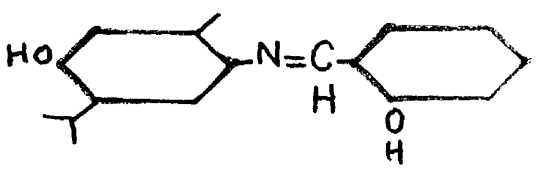
solution. To this was added 2.72 g. (0.02 mole) of powdered sodium acetate, a portion of which dissolved. The mixture was then mechanically stirred and to it was slowly added 4 g. (0.02 mole) of p-aminothymol hydrochloride previously dissolved in 22 ml. of dehydrated alcohol. A cream-colored precipitate began to form almost immediately and the stirring was continued after all of the amine had been added, until most of the alcohol had evaporated. The cream-colored solid was filtered off by suction and the filtrate added to several volumes of cold water to give some additional precipitate. The precipitates were combined, washed well with water and placed in a desiccator over calcium chloride. It should be stated here that in two attempts we were unsuccessful in condensing p-aminothymol and piperonal either in aqueous or alcoholic mediums without the use of a weak alkali.

The compound was prepared three times by the method outlined above for an average yield of 75% of the theoretical. The cream-colored solid was recrystallized from 60% ethyl alcohol to give light yellow slightly iridescent scales which melted at 161-162°C. (corr.). Under the conditions which prevailed during the experiment this Schiff's base did not undergo the marked color changes so common to certain other compounds of this type.

Analyses: Calculated for $C_{18}H_{19}O_3N$ N = 4.71

Found N = 4.56

2-HYDROXYBENZYLIDENE-4-AMINOTHYMOL. The aldehyde from



which this Schiff's base was prepared, namely, salicylaldehyde (o-hydroxybenzaldehyde), has been studied probably more extensively than any other with a view toward synthesizing organic medicinals.[18] This is doubtless due to the fact that it is oxidizable to salicylic acid, a compound of major importance in therapeutics.

Six grams (0.05 mole) of salicylaldehyde was added to 150 ml. of water in which was previously dissolved 6.8 g. (0.05 mole) of sodium acetate. To this was added 10 g. (0.05 mole) of finely powdered p-aminothymol hydrochloride. The mixture was mechanically shaken for three hours during which time the Schiff's base precipitated as a copious orange-colored solid which was filtered off by suction, washed with water and then thoroughly dried in a desiccator over calcium chloride.

It was found that when the aromatic aldehyde to be condensed with p-aminothymol was in a liquid state the best yields resulted when the above procedure was used. The yield in which this particular compound was obtained was above 95% of the theoretical.

This Schiff's base occurred in both a yellow and red modification but since the red form showed greater stability, the desmotropes were more difficult to isolate than those of the corresponding compound obtained from p-homosalicylaldehyde with p-aminothymol.

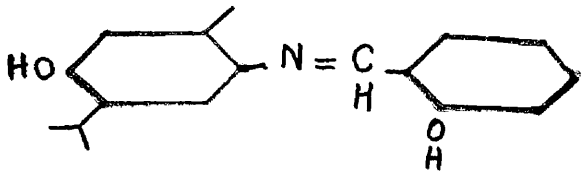
This compound was recrystallized from benzol as fine yellow needle-shaped crystals as well as from 60% ethyl alcohol. When recrystallized from the diluted alcohol it was recovered as a very fine orange-colored powder which melted at

170°C. (corr.). The loss in weight of a sample of the compound dried for one hour at 110°C. was negligible.

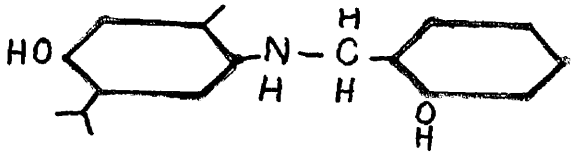
Analyses: Calculated for $C_{17}H_{19}O_2N$ N = 5.20

Found N = 5.12

In an attempt to reduce this Schiff's base

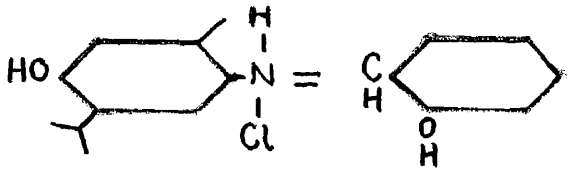


(with hydrogen catalyzed by platinum) to the secondary amine,



the reaction mixture on exposure to air became purple and moreover possessed a strong phenolic odor which was found to be very similar to that of a pure sample of ortho-cresol. From this evidence we suspected that the Schiff's base molecule had undergone fission at the C = N linkage. This we were able to confirm by a subsequent reduction of the compound from which was isolated 70% of the calculated amount of p-aminothymol as its hydrochloride, the identity of which was established by a mixed melting point determination.

The hydrochloride of this Schiff's base,



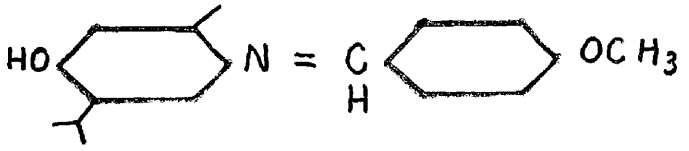
(2-hydroxybenzylidene-4-aminothymol hydrochloride) was prepared in this way:

The Schiff's base 1.36 g. (0.005 mole) was dissolved in

250 ml. of benzol and dry hydrogen chloride passed into this solution as long as any precipitate was formed. The precipitate was yellow whereas the original Schiff's base was orange-colored. The yellow precipitate on contact with water immediately assumed its orange color. This may be explained on the theory that the orange-colored modification shifted to the yellow desmotrope, a compound more easily salified by the acid. Then on contact with water, hydrogen chloride was split off of this molecule which allowed the compound to assume its more stable orange-colored form.

A 0.1022 g. sample of the Schiff's base hydrochloride lost 0.0128 g. while standing for fifteen hours in a desiccator connected with a good vacuum pump while the calculated loss for hydrogen chloride was 0.0122 g.

4-METHOXYBENZYLIDENE-4-AMINOTHYMOL. Anisaldehyde



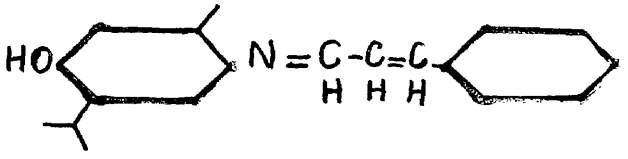
(p-methoxybenzaldehyde) the aldehyde from which this Schiff's base was prepared, has been used as a perfume but not as a therapeutic agent as far as could be determined.

To 100 ml. of water containing 2.72 g. (0.02 mole) of sodium acetate was added 2.64 g. (0.02 mole) of anisaldehyde. This was shaken until the water-insoluble anisaldehyde was well distributed and then to this mixture was added 4 g. (0.02 mole) of aminothymol hydrochloride. The mixture was then mechanically shaken until all of the oily drops of anisaldehyde had disappeared and a copious white precipitate had

formed. The precipitate was filtered off, dried on porous plate, and then recrystallized from carbon tetrachloride to yield practically colorless crystals which, viewed under the low power of a microscope, appeared to be oblong scales. These crystals melted at 160°C. (corr.). A yield of 5.7 g. was obtained which represented 95% of the calculated amount. This Schiff's base did not undergo color changes when it was recrystallized or melted nor on standing in diffused sunlight.

Analyses:	Calculated for $C_{18}H_{21}O_2N$	N = 4.94
	Found	N = 4.74

CINNAMYLIDENE-4-AMINOTHYMOL. Cinnamylaldehyde was



included among the aldehydes used in this study for two reasons. First, its physical properties[78] and physiological properties[79] are similar to those of the true aromatic aldehydes. Second, since cinnamylaldehyde is a vinylog of benzaldehyde, it was of interest to determine whether it could be condensed with p-aminothymol to produce the corresponding Schiff's base in yields which approached those obtained with p-aminothymol and benzaldehyde. The influence of vinylogy on the condensation was indicated by these facts. It was found that cinnamylaldehyde condensed smoothly with p-aminothymol whereas attempts to condense hydrocinnamylaldehyde,

the dihydro derivative of cinnamylaldehyde with p-aminothymol were not very successful.

Two grams (0.01 mole) of p-aminothymol hydrochloride was dissolved in 10 ml. of methyl alcohol and this solution was then added to 1.32 g. (0.01 mole) of freshly distilled cinnamylaldehyde previously dissolved in 30 ml. of methyl alcohol to which had been added 1.36 g. (0.01 mole) of sodium acetate. A gentle stream of carbon dioxide was passed through the solution during which time the alcohol was allowed to evaporate slowly. This gave a good yield of a dark orange-colored powder which was freed of sodium chloride, acetic acid and other impurities by washing it well with water.

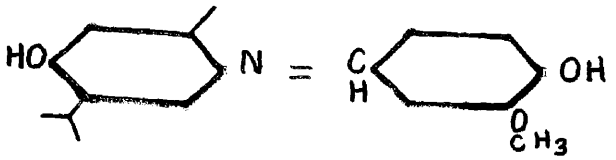
This Schiff's base like the others prepared with p-aminothymol and a liquid aromatic aldehyde can likewise be obtained in good yields by agitating equivalent amounts of p-aminothymol hydrochloride, cinnamylaldehyde and sodium acetate in a moderate amount of water. When prepared by this procedure it first appears as a red, viscid, immiscible liquid which crystallizes with no great difficulty.

This Schiff's base was best recrystallized from relatively small amounts of ethylene dichloride to give orange-colored prisms melting at 154°C. (corr.). These orange-colored crystals became distinctly yellow when they were powdered by trituration in a mortar. The loss in weight of a sample of the compound at 90°C. for one hour was negligible which indicated no solvent of crystallization. The yield in which this Schiff's base was obtained depended on the procedure by which it was prepared. Of the two procedures

used, the one employing agitation of the reactants in an aqueous medium gave the smaller yield which was 70% of the theoretical amount.

Analyses:	Calculated for $C_{19}H_{21}ON$	N = 5.01
	Found	N = 4.88

3-METHOXY-4-HYDROXYBENZYLIDENE-4-AMINOTHYMOL.



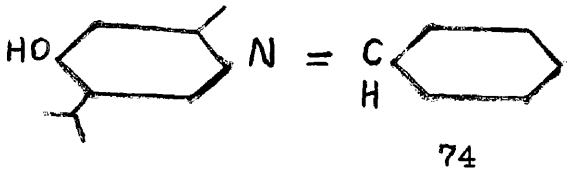
Vanillin or the methyl ether of protocatechuic aldehyde which was used in preparing this Schiff's base has previously been condensed with p-ethoxyaniline to prepare another Schiff's base which is credited with having styptic properties.[80]

Vanillin 1.50 g. (0.01 mole) was dissolved in 200 ml. of distilled water. In this solution was also dissolved 2 g. (0.01 mole) of p-aminothymol hydrochloride. At this point the solution became yellow but showed no sign of precipitation. Sodium bicarbonate 0.84 g. (0.01 mole) was added to the solution in divided portions the solution being shaken after the addition of each portion. Soon after the first portion of sodium bicarbonate was added an almost white precipitate began to form which increased as more sodium bicarbonate was added. Before all of the sodium bicarbonate had been added the mixture became purple due to the oxidation of some of the free p-aminothymol. The precipitate was filtered off, washed well with water and then dried on a porous plate. The precipitate was recrystallized three times from chloroform to yield 2.4 g. of light yellow blade-shaped crys-

tals which melted at 194°C. (corr.). A yield of 2.4 g. represented 83% of the theoretical amount. This Schiff's base did not show marked phototropism, but an alkaline alcoholic solution of it did become dark red on standing.

Analyses:	Calculated $C_{18}H_{21}O_2N$	N = 4.90
	Found	N = 4.80

BENZYLIDENE-4-AMINOTHYMOL. This Schiff's base has



been prepared previously by Plancher[74] who obtained the compound by heating freshly distilled benzaldehyde and p-aminothymol for two hours at a temperature of 120-150°C. It seemed desirable to prepare this compound to complete the series of Schiff's bases which were being studied both from a chemical and pharmacological standpoint.

This Schiff's base was obtained in an analogous manner to the others which were prepared from a liquid aromatic aldehyde and p-aminothymol, i.e. by shaking equivalent amounts of benzaldehyde and p-aminothymol hydrochloride in water in which had been dissolved an equivalent amount of sodium acetate. A practically quantitative yield of benzylidene-4-aminothymol was obtained by this method.

The compound was recrystallized from benzol to give practically colorless plates which melted at 148°C. (uncorr.). Plancher[74] recorded the melting point as 148-150°C. This Schiff's base did not undergo color changes under the conditions employed in its preparation, purification and identi-

fication.

PART IV

PHARMACOLOGICAL

Something of the nature and degree of pharmacological activity which thymol possesses was given in the introductory portion of this thesis. As far as could be determined there are no published data concerning the pharmacological behavior of p-nitrosothymol and p-aminothymol, the two intermediates from which were made the six new Schiff's bases just described.

The study which we made of these compounds consisted principally of a preliminary investigation of their relative antipyretic activity. This activity was determined by administering the compounds in capsules to cats. The rectal temperature of the animal was taken before administration of the compound and at stated intervals thereafter by means of a lubricated rectal thermometer.

p-Nitrosothymol administered to a cat in a dose of 0.02 g. per Kg. was without noticeable effect on the size of the pupil of the cat's eye. The animal was observed for three hours after administration of the compound but no change in body temperature, erection of hair nor vomiting could be noted. An examination twenty-four hours later as well as one ten days later showed the cat to be alive and healthy.

A second cat which had received 0.02 g. of p-aminothymol hydrochloride per Kg. vomited after forty-five minutes.

Except for this the animal appeared normal and it is possible that the emesis was caused by the acidity of the compound. An examination of this cat the next day and one week later revealed the animal to be alive and apparently in good condition.

Benzylidene-4-aminothymol administered in a dose of 0.02 g. per Kg. to a cat whose rectal temperature was 102.2°F. lowered the temperature of the animal to 101.5°F. within one hour. The compound appeared not to produce other reactions in the animal and subsequent examinations showed the animal to be normal.

4-Methoxybenzylidene-4-aminothymol administered in a dose of 0.02 g. per Kg. to a cat whose rectal temperature was 102°F. lowered the temperature of the animal to 101°F. within one hour. No other changes as erection of hair, change in the size of the pupil nor vomiting were observed in the cat and it appeared normal throughout an observation period of ten days.

2-Hydroxybenzylidene-4-aminothymol was exceptionally active as an antipyretic in cats. An animal whose temperature was 102.7°F. prior to administration of the compound in a dose of 0.02 g. per Kg. had a temperature of 98.7°F. within one hour after the compound was given. As in the case of the other compounds this cat showed no ill effects from the dose indicated.

Since 2-hydroxybenzylidene-4-aminothymol appeared to be active as an antipyretic its toxicity was ~~as~~ compared to that of acetanilid on the cat. It was shown that 0.25 g. of

acetanilid per Kg. was the lethal dose with death usually resulting within eighteen hours. 2-Hydroxybenzylidene-4-aminothymol was given to cats in doses as high as 1 g. per Kg. without noticeable effect on the animal. The cat was observed closely for three hours after the compound was administered during which time it appeared normal. Apparently there was no hypnotic effect produced. The animal was alive the next day as well as ten days later and in good condition on both occasions.

SUMMARY

The tautomerism of the p-nitrosothymol-thymoquinone-monoxime system was studied with a view of the effect of this on the hydrolysis of the compound.

Thymoquinone was obtained in fair yields by hydrolyzing p-nitrosothymol by means of a catalyst in an acid solution.

p-Nitrosothymol was quantitatively reduced to p-aminothymol by the use of hydrogen in the presence of a palladium catalyst as well as a platinum catalyst.

p-Aminothymol was diazotized and thymohydroquinone was obtained from the resulting diazonium salt in good yields by a new method.

Six new Schiff's bases with p-aminothymol were prepared and described.

A preliminary pharmacological study of p-nitrosothymol, p-aminothymol and several Schiff's bases was begun and something of the comparative toxicity of 2-hydroxybenzylidene-4-aminothymol to acetanilid was determined.

The marked antipyretic activity and comparative low toxicity of the Schiff's base from p-aminothymol and salicylaldehyde warrants further pharmacological investigation of this compound.

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A STUDY OF NITROSOTHYMOL AND AMINOTHYMOL WITH PARTICULAR REFERENCE TO SCHIFF'S BASES OF AMINOTHYMOL

June 1939

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