The Synthesis of Potential Antimalarials

I. Analogues of Pantothenic Acid

II. N^1-Phenylsulfanilamides

III. Sulfonamidopyrimidines

IV. Quinolinemethanols Related to Quinine

The Effect of Globulin Depletion on Antibody Production in Rabbits

Thesis

by

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In Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

California Institute of Technology

Pasadena, California

1946

ACKNOWLEDGMENT

I would like to express my appreciation to Dr. J. B. Koepfli for directing the research described in this thesis and for guiding my graduate work. I also wish to thank my colleagues on the antimalarial program at the California Institute of Technology for a great deal of advice and assistance and, especially, Dr. Maurice Rapport, who collaborated with me in several of the syntheses described. Dr. Dan Campbell suggested the problem for my minor research and patiently supervised the experimental work.

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GENERAL INTRODUCTION

Soon after the start of the war with Japan in 1941 it became apparent that one of the most pressing medical problems facing the United States was the control of malaria. The early conquests by Japan in the Dutch East Indies interferred with the use of quinine for the control of this disease, since the major world production centers of the alkaloid were under enemy control. At the same time it was obvious that much of the fighting would take place in highly malarial regions, especially in the South Pacific Ocean.

The synthetic drugs which were available for use against malaria were relatively new and untried. Atebrin was very promising but there were various unpleasant effects attending its use, and it was not certain that it could completely replace quinine. Plasmochin, although extremely active, was of little use as a general drug, due to its great toxicity. Neither of these drugs, nor for that matter quinine itself, acted as a causal prophylactic, a property which would have been extremely desirable in a drug.

In this emergency the Committee on Medical Research of the Office of Scientific Research and Development sponsored a large program of research on malaria. One branch of this program was concerned with testing a large number of drugs for possible antimalarial activity, in the hope of finding new classes of active compounds, of discovering more active members of classes of known activity, and possibly of finding a causal prophylactic. To assist this phase of the program the Survey on Antimalarial Drugs was set up to test the available compounds. Most

of the drugs tested were examined for activity against avian malaria, although certain compounds which showed exceptional promise were tried out in human malaria. The two tests used most frequently were for activity against P. lophurae in ducks, and against P. gallinaceum in chicks.

Many of the drugs which were tested in this program were compounds immediately available, from the shelves of chemical manufacturers, pharmaceutical houses, etc. In addition a great deal of work in synthetic organic chemistry was sponsored to provide new drugs for testing. This thesis describes a number of syntheses carried out by the author between September 1942 and December 1945, working under a contract between the Office of Scientific Research and Development and the California Institute of Technology. This project was under the direction of Dr. Joseph B. Koepfli.

Being carried out as part of a war research program, this work differs in several respects from the type of work usually described in a thesis. Since the primary object was to prepare the desired compounds as quickly as possible in a given quantity, it was not always necessary to examine reactions critically for conditions of maximum yield and ease of working. If a reaction took place with a low yield, it was often quicker to carry it out several times than to try to find conditions under which the yield might be improved. In several cases interesting side reactions were noticed; these were ignored unless the by-products formed seemed to have a direct bearing on the problem at hand.

In addition the work often took on a cooperative aspect, with several members of the project working simultaneously on the same problem and even, on occasion, on the same reaction. In so far as possible I have endeavored

to acknowledge assistance with particular problems in the body of this report.

The details of the pharmacological action of the compounds whose preparation is described are not discussed. It is the intention of the Survey on Antimalarial Drugs to publish a monograph in which the activities of all the compounds tested will be given. The number designated by SN given following the compounds in this thesis is the number by which these compounds will be designated in the report of the Survey.

I. THE PREPARATION OF PANTOTHENIC ACID INHIBITORS AS POTENTIAL ANTIMALARIALS

INTRODUCTION

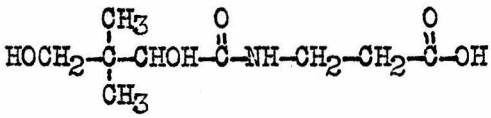
The theory that the action of certain drugs, which are active against bacterial infections, is due to interference by the drug with some essential metabolic process of the bacteria is one that has gained general acceptance in recent years. This theory was discussed by Fildes[1], and was based in part on the work which had been done on the mode of action of sulfanilamide.

Woods[2] had demonstrated that the <u>in vitro</u> activity of sulfanilamide can be reversed by adding para-aminobenzoic acid to the medium, and suggested that para-aminobenzoic acid was the active principle, isolated from bacterial cells by Stamp[3] and Green[4], and by himself from yeast, which was able to reverse the bacteriostatic action of sulfanilamide. Fildes and Wood postulated that para-aminobenzoic acid was an essential metabolite for the bacteria affected by sulfa drugs. They pictured the activity of these compounds as arising from their ability to react with some enzyme in the bacterial cell for which para-aminobenzoic acid was the normal substrate, thus blocking the metabolic process in which that enzyme system took part, and so preventing growth. This ability to react with the enzyme was considered to be based on the structural similarity between sulfanilamide and para-aminobenzoic acid. Since the reaction between the enzyme and the sulfonamide on the one hand, and the

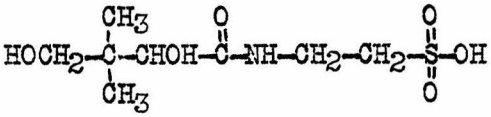
enzyme and para-aminobenzoic acid on the other, was a competetive one, adding more para-aminobenzoic acid reversed the former reaction and permitted growth.

Fildes had suggested that future work might be carried out along the line of altering the molecules of other known bacterial metabolites, in order to find new examples of this type of action, and possibly to develop new drugs. McIlwain then demonstrated a similar type of competition between pyridine-3-sulfonic acid and the bacterial metabolite nicotinic acid[5], and between a group of α-aminosulfonic acids, and the naturally occurring amino acids.[6]

Since pantothenic acid, α,γ-dihydroxy-β,β-dimethylbutyryl-β-alanine (I), was known to be a growth factor for several bacteria various analogues of it were tested as bacterial growth inhibitors. Snell[7] prepared pantoyltaurine* (II), the sulfonic acid analogue of pantothenic acid, and showed it inhibited the growth of Lactobacillus arabinosis, and that the inhibition could be reversed by adding pantothenic acid to the medium. The inhibitor, pantoyltaurine, showed the same stereochemical specificity as pantothenic acid itself, since the dextro form was much more active than the laevo form.



I



II

*The simplified nomenclature suggested by Barnett and Robinson[10] is used in this thesis. In this α,γ-dihydroxy-β,β-dimethylbutyric acid is given the trivial name of pantoic acid.

Similar results were obtained by Kuhn, Wieland, and Möller[8] in Germany, and by McIlwain[9] in England. McIlwain tested a number of analogues of pantothenic acid, prepared by Barnett and Robinson[10], and found pantoyltaurine to be the most active inhibitor.

Up to this time all the known cases of specific inhibition by a metabolite analogue had been demonstrated with bacteria. It had not been possible to discover any essential metabolites for protozoan parasites. However Trager[11] developed a method by which it was possible to cultivate the malarial plasmodium in vitro. With a view to finding some essential metabolite for these parasites he studied the effect of adding various compounds, known to be of importance in bacterial metabolism, to his synthetic medium. He found[12] that the addition of calcium pantothenate to the culture resulted in lengthening the survival time of the parasites from six days to about fifteen days. While the amount added, 0.02 mg./ml., is much greater than that which would be found in the blood stream of ducks or chicks, it was indirect evidence that pantothenic acid functions as a plasmodial metabolite.

Based on this result Trager suggested the testing of a number of analogues of pantothenic acid for possible activity as antimalarials. Accordingly a large number of compounds related to pantothenic acid was prepared by Dr. Koepfli's group and submitted for test. These could be divided into several classes, the first consisting of pantoyltaurine, pantoyltauramide, and N-substituted pantoyltauramides. The

preparation of one of the latter compounds, pantoyltauryl-2-amino-pyrimidine, is described in the succeeding section of this thesis. The second class consisted of corresponding compounds with the pantoyl group replaced by the γ-hydroxybutyryl rest. γ-Hydroxybutyryl-tauryl-2-aminopyrimidine is described in the following pages. In addition a number of miscellaneous compounds were prepared, with other types of blocking groups on taurine, other alterations of the β-alanine portion of pantothenic acid besides that to taurine, and combinations of both of these.

The method used for testing these compounds for antimalarial activity was later found to have been most unfortunate. P. lophurae was the parasite used by Trager in his studies, and seemed to be the logical one to try. All the compounds tested were inactive against infections of this parasite in ducks. Pantoyltauramide was tested against P. gallinaceum in chicks. Since it was not adsorbed unaltered after feeding it was given by intravenous injection. It showed definite antimalarial activity, leading to complete suppression of parasitemia after several days. It was given at high dosage levels, as much as 2000 mg./kg. body weight, but was so completely non-toxic that this could be done without difficulty. In later work it was shown that some other pantothenic acid analogues were effective when given to chicks by mouth. Investigation of this phenomenon led to the conclusion that this was a case of host specificity towards the action of the drugs. Duck infections seem to be completely unaffected by compounds of this type which are active in chicken malaria. That this is a true

host specificity and not a species specificity of the parasite is shown by the fact that in chicks P. lophurae is as susceptible to these drugs as P. gallinaceum. It is tempting to conjecture that this may be connected with a higher concentration of pantothenic acid in ducks than in chicks; there is no direct evidence on this point.

The therapeutic action of compounds of this class is a true case of inhibition. This can be shown since the suppressive effect of pantoyltauramide, as well as of the drugs to be mentioned directly, can be reversed by feeding the test birds excess amounts of pantothenic acid during the experiments. Hence, pantoyltauramide is the first compound which has been shown to have a specific inhibitory effect upon protozoan metabolism.

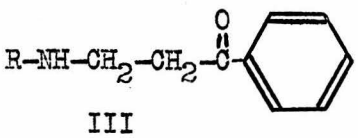
It can be seen from the above remarks that it is unfortunately true that most of the compounds which were prepared in this work were tested in a manner which would not have shown any activity they might have possessed. It seems likely, in view of the activity of pantoyltauramide, that several of its N-substituted derivatives might prove to be active if they were retested against P. gallinaceum in chicks.

On the basis of the disappointing results of the tests against P. lophurae, research along these lines was dropped, in order to concentrate on more promising classes of compounds. At a later time the work was revived on the basis of some new evidence. The American Cyanamide Company, which had been interested in this problem at the time of our original work, had continued to try to find some pantothenic acid analogue which could be readily adsorbed when given by mouth.

They tested the N-phenyl derivative of pantoyltauramide, pantoyltauryl-anilide, and found it to have a strong activity in chick malaria. Since a chlorine in the para position in several classes of antimalarials increases activity they tried pantoyltauryl-p-chloroanilide and found it to be extremely active.

Since the original work on pantothenic acid inhibition, a number of compounds besides pantoyltaurine derivatives had been shown to be able to act as inhibitors towards bacteria. Thus Pollack[13] found that α-methylpantothenic acid, which could act as a substitute for pantothenic acid to a small extent in the absence of the latter, acted as an inhibitor in the presence of pantothenic acid. Barnett[14] prepared N-pantoyl-β-aminoethylthiol, bis-(pantoyl-β-aminoethyl)-sulfide, -disulfide, -sulfoxide, and -sulfone and showed that they all acted as inhibitors. And Snell and Shive[15] showed that the alcohol corresponding to pantothenic acid, as well as several structurally similar alcohols acted as inhibitors. Then Woolley[16] published a review in which he discussed the production of symptoms of vitamin deficiencies by feeding analogues of vitamins to animals. He mentioned the compound phenyl pantothenone (III), pantoyl-β-aminoethylphenyl ketone. Dr. K. C. Blanchard noticed this and suggested that the compound be tested as an antimalarial; it proved to have definite activity. Since then a number of compounds related to Woolley's ketone have been prepared elsewhere and tested. Several of them show activity against malaria in chicks.

Dr. Koepfli suggested preparing the compounds IV–VIII shown below, some of which may be considered to be sulfur analogues of Woolley's ketone. Their synthesis is described in the following portion of the thesis. After the work was commenced our attention was called to the paper of Madinaveitia *et al*[17] in which several N-pantoyl-β-aminoethylphenylsulfones were prepared by similar methods; none of these duplicated our compounds.



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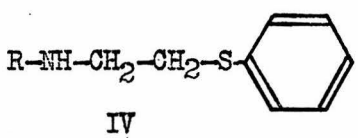

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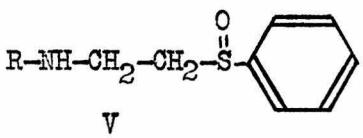

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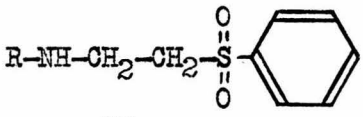

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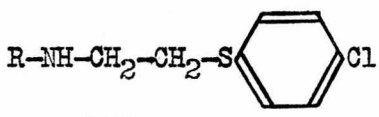

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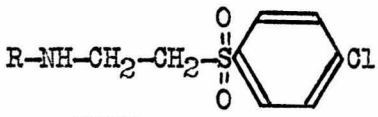

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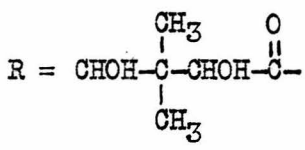


The three unsubstituted phenyl compounds IV, V, and VI have been tested against P. gallinaceum in chicks, being given by mouth, and showed activities from one to two times that of quinine. At the present time the test results on the two chlorinated compounds VII and VIII have not been received; in view of the great activity of a chlorine in this position in other series, it is to be hoped that they may be more active yet.

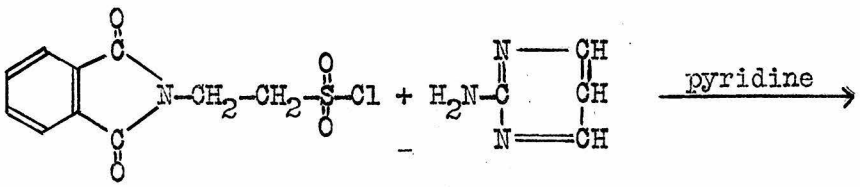
This work is important as it represents the first extension of the theory of inhibition of an essential metabolite to protozoa, and also because it is an example of the rare phenomenon in chemotherapy of the

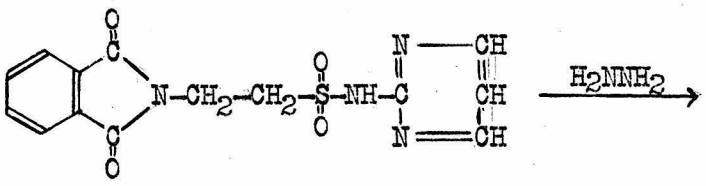
prediction of the activity of a new class of drugs against a specific disease in advance of pharmacological testing. Whether this type of compound will attain any practical importance it is too early to say. It is not known if man will tend to behave as the chick or as the duck when treated with compounds of this type; if the latter animal is the prototype, then the drugs will be useless. There are also certain chemical drawbacks to these compounds, although these are perhaps of a minor nature. Many of them tend to form viscous sirups which are hard to crystallize; on the other hand, some have been successfully crystallized and are easy to handle. While the amino portions of the molecules are generally fairly easy to prepare, the l-pantolactone required for their preparation is extremely expensive, although it is commercially available.

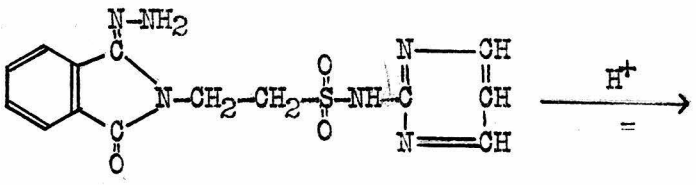
EXPERIMENTAL

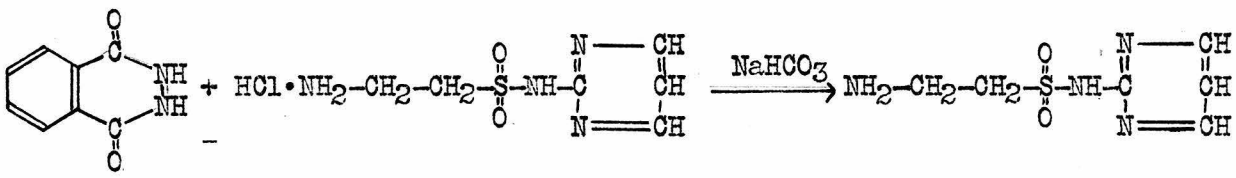
The Preparation of Tauryl-2-aminopyrimidine Hydrate

This compound was prepared by the reactions shown in the equations below. This general method of preparing derivatives of tauramide is based on the preparation of tauramide hydrochloride by Miller, Sprague, Kissinger, and McBurney[18], and has been used in this laboratory to prepare a large number of tauramide derivatives. The condensation between 2-phthalimidoethanesulfonylchloride and 2-aminopyrimidine occurred on mixing the components at room temperature in pyridine;









higher temperatures caused extensive decomposition of the material. This was in accord with the observation by Roblin *et al*[19] on the necessity of low temperatures during the reaction between 2-aminopyrimidine and sulfonyl chlorides. The removal of the phthalyl rest by the Ing-Manske procedure went smoothly and gave a good yield of tauryl-2-aminopyrimidine hydrochloride. The only difficulty was experienced when an attempt was made to convert the hydrochloride to the

free base. The usual procedure used in carrying out this type of reaction was to dissolve the tauramide hydrochloride in question in water, make the solution basic with sodium bicarbonate, remove the water in vacuo, and take the hygroscopic free base up in ethanol. With this particular compound neutralization of the amine hydrochloride in an aqueous solution caused an immediate precipitate of crystalline solid. This was first assumed to be the free base, but the analytical results and the difficulties in reacting it with the pantoyl lactone described in the succeeding section forced the conclusion that it was a hydrate.

2-Phthalimidoethanesulfonyl-2-aminopyrimidine. - In 50 ml. of anhydrous reagent pyridine was suspended 17.2 g. of 2-aminopyrimidine* and 46.5 g. of 2-phthalimidoethanesulfonylchloride[18] was added in small portions. The solid slowly dissolved and after an hour a flocculent precipitate began to form. The suspension was stirred overnight and then poured into 1 l. of water. The resulting mixture was brought to neutrality with sodium bicarbonate and the solid product was filtered off. After drying a brown powder weighing 34.3 g., 57%, was obtained, melting at 249-254°**. Dr. Mead prepared an analytical sample by recrystallization from acetic acid which melted at 249-251°.

Anal. Calcd. for $C_{14}H_{12}N_4O_4S$ (332.3): C, 50.6%; H, 3.6%.
Found: C, 50.5%; H, 3.7%.

*This was kindly given to us by the American Cyanamide Company.
**All melting points reported in this thesis are corrected for thermometer deviations and for exposed stem.

Tauryl-2-aminopyrimidine hydrochloride. - In 200 ml. of refluxing 95% ethanol was suspended 25.6 g. of the crude product from above, and 15 ml. of 42% hydrazine hydrate was added. The solid dissolved and in about ten minutes the white hydrazine addition product began to come out of solution. After refluxing one hour the mixture was chilled and the precipitated addition product filtered off. The addition of 20 ml. of concentrated hydrochloric acid now caused an immediate precipitation of phthalhydrazide. The mixture was cooled and this latter compound filtered off. The filtrate was concentrated in vacuo to 75 ml. and 1 l. of 95% ethanol was added. This threw down a fine yellow solid weighing 9.3 g. and melting at 214-215°. The addition of ethyl ether to the mother liquors gave a second crop of 4.6 g., giving a total yield of 13.9 g., 75%. A sample of the product recrystallized several times from aqueous ethanol was colorless and melted at 217-218°. It gave the following analysis.

Anal. Calcd. for $C_6H_{11}N_4O_2SCl$ (238.7): C, 30.19%; H, 4.64%; N, 23.48%.
Found: C, 30.48%; H, 4.47%; N, 23.86%.

Tauryl-2-aminopyrimidine hydrate. - Fourteen grams of the hydrochloride was dissolved in 60 ml. of water. One normal sodium bicarbonate solution was added till the evolution of carbon dioxide ceased. During the addition a crystalline precipitate formed which was filtered off and dried in a vacuum desiccator. It weighed 9.5 g., 73%, and melted at 151-153° with the evolution of gas and decomposition to a brown melt, after sintering and softening from about 140°. Several recrystallizations from aqueous alcohol did not change this abnormal behavior. An analytical sample, which

was prepared by Mr. Rapport by recrystallization from water, melted with effervescence at 151°.

Anal. Calcd. for $C_6H_{10}N_4O_2S$ (202.2): C, 35.64%; H, 4.98%; N, 27.71%.
for $C_6H_{10}N_4O_2S \cdot H_2O$ (220.3): C, 32.72%; H, 5.49%; N, 25.44%.
Found: C, 32.95%; H, 5.12%; N, 25.31%.

The Preparation of γ-Hydroxybutyryltauryl-2-aminopyrimidine

Two general procedures have been described in the literature for carrying out condensations of pantolactone with amines to give amides. One is the neat condensation of the two reactants at an elevated temperature, and was first employed by Williams[20] in his initial synthesis of pantothenic acid. The other, introduced by Reichstein[21], employs a solvent with lower temperatures and a longer period of heating. Both methods have been used extensively by the various authors mentioned in the introduction to this section for the preparation of analogues of pantothenic acid. The former method has given good results in the majority of cases investigated in this laboratory and was used here.

The material from this reaction was not crystallized and some doubt may exist as to whether the desired compound was obtained, especially in view of the difficulties later encountered in preparing the pantoyl compound. The fact that the fusion mixture was completely soluble in absolute ethanol seemed to indicate substantial completion of the reaction, since one of the starting materials, tauryl-2-aminopyrimidine hydrate, was insoluble in this solvent. The high yield obtained served as

confirmation, since any unreacted lactone should have been removed by the ether extraction giving a smaller amount of oil.

γ-Hydroxybutyryltauryl-2-aminopyrimidine (SN 3276). - In a test-tube were placed 5.63 g. of tauryl-2-aminopyrimidine hydrate and 3.87 g. of γ-hydroxybutyrolactone. The tube was heated in a water bath at 100° for six hours, during which time the solid slowly liquified giving a thick oil. After cooling the oil was extracted thoroughly with ethyl ether to remove unreacted lactone, and then dissolved in hot absolute ethanol. On cooling no solid came out, indicating the absence of any unreacted amine hydrate which is extremely insoluble in ethanol. The ethanol was removed *in vacuo* to give a pale yellow oil which was dried to constant weight in a vacuum desiccator. It weighed 7.6 g., 102%, and was hygroscopic. For biological testing it was made up in an aqueous solution containing 333 mg. of amide/ml. of solution.

The Preparation of d-N^2-(Pantoyltauryl)-2-aminopyrimidine*

This compound was prepared by the method of direct condensation between l-pantolactone** and tauryl-2-aminopyrimidine hydrate, before the latter was shown to be a hydrate. A poor but substantial yield of the desired product was obtained. Attempts to repeat this reaction on a larger scale led to total failure, as it was possible to isolate from subsequent runs only hygroscopic, filmy solids, or else the byproduct mentioned below. Because of this the synthesis was

*The letters d and l are used only to denote the sign of the rotation of polarized light by the compounds, and do not imply anything about the configuration.

**This lactone was given to us by Merck and Company.

temporarily dropped, in so far as preparing material for test was concerned.

The problem was subsequently reinvestigated by Mr. Rapport, and on the basis of his results it is possible to explain the difficulty of repeating the initial synthesis. He showed that condensation could only occur between the lactone and the free base after the latter had lost its water of hydration. Since dehydration of the amine led to its partial decomposition it was necessary to carry out the dehydration under strictly controlled conditions. His method of synthesis called for dehydrating the base at a given temperature for a stated period of time which caused partial dehydration, followed by condensation of the partly decomposed material with pantolactone. In one instance he isolated some product from a fusion mixture of amine hydrate and l-pantolactone, but the yield was very small. Evidently the production of the desired material in such a case depends very critically on the conditions of heating.

In several of the reactions which failed to give the desired product there was isolated an interesting byproduct which melted at the same point as tauryl-2-aminopyrimidine hydrate, without the decomposition characteristic of the latter's melting. The best empirical formula from the analytical data was that of tauryl-2-aminopyrimidine, but it failed to give the strong ninhydrin reaction which was given by all the tauramide derivatives prepared.

d-N^2-(Pantoyltauryl)-2-aminopyrimidine (SN 7293). - Six grams of tauryl-2-aminopyrimidine and 4.50 g. of l-pantolactone were mixed well and heated for one hour at 130-135°. The mass became liquid. During this period the temperature of the bath was raised to 145° for several minutes, and a gas was evolved which caused anhydrous copper sulfate to turn blue. For two and one-half additional hours the mass was heated at 100°.

The melt was taken up in 20 ml. of ethanol, and the solution poured into 250 ml. of dry acetone, precipitating a quantity of white, gummy material. This was removed by filtration, and appeared to be very hygroscopic and sensitive to air. The mother liquors on treatment with ether yielded a little more of this gum. A second addition of ether to the mother liquors caused a white crystalline solid to come out slowly, which proved to be d-N^2-(pantoyltauryl)-2-aminopyrimidine. It weighed 1.44 g., a yield of 16%. A sample after several recrystallizations from an ethanol-isopropyl ether mixture melted at 177.5-178.5°.

Anal. Calcd. for $C_{12}H_{20}N_4O_5S$ (332.4): C, 43.36%; H, 6.07%; N, 16.86%.

Found: C, 43.76%; H, 5.89%; N, 16.34%.

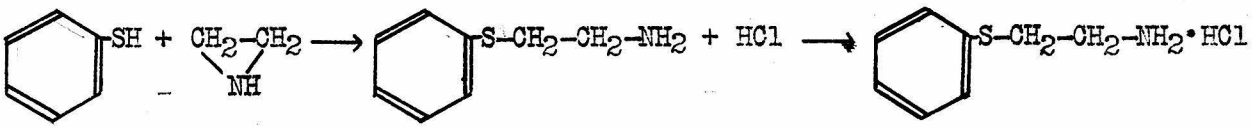
$[\alpha]_D^{23°} = 23.6°$ (21.9 mg. in 1.99 ml. of water).

When attempts to repeat this synthesis failed the condensation was tried using methanol, ethanol, cellosolve, butanol, dioxane, acetonitrile, and pyridine as solvents. With each solvent except pyridine no reaction occurred; with pyridine it was impossible to isolate anything from the dark-colored solution obtained.

In subsequent attempts at neat condensations the only material which could be isolated in a crystalline condition was a white solid, m.p. 150-153°, which resembled the desired product in its method of isolation and solubility properties. An aqueous solution did not rotate the plane of polarized light. An analytical sample gave C, 36.4%; H, 5.16%; N, 25.53%. Calculated for tauryl-2-amino-pyrimidine, $C_6H_{10}N_4O_2S$ (202.2): C, 35.6%; H, 4.99%; N, 27.7%. This compound gave no test with ninhydrin and sodium acetate, while tauryl-2-aminopyrimidine hydrate gave a strong purple color.

The Preparation of d-N-Pantoyl-β-aminoethylphenylsulfide

This compound was prepared in the usual fashion by a neat condensation between l-pantolactone and β-aminoethylphenylsulfide. The hydrochloride of the latter compound has been described in the literature by Gabriel and Colman[22], who prepared it by condensing β-bromoethylphthalimide with sodium thiophenolate, and hydrolysing off the phthalyl rest. The synthesis described below, which is much quicker and simpler, was suggested by the preparation by Barnett[14] of β-aminoethylmercaptan by a similar reaction between ethylenimine and hydrogen sulfide.



The product of the condensation was an oil which was impossible to crystallize. It was purified by a method similar to that employed by Woolley[23] in his synthesis of phenyl pantothenone, that is solution in an organic solvent and extraction with water and dilute acid to remove unreacted starting materials. The analysis and rotation obtained on the oil subsequent to removal of the solvent indicate that it was essentially pure.

β-Aminoethylphenylsulfide hydrochloride. - Ethylenimine was prepared as described by Wenker[24]. To a solution of 77.0 g. of thiophenol in 300 ml. of absolute ethanol was added dropwise 30.0 g. of ethylenimine over a five-minute period. The solution was cooled in an ice bath as the reaction generated a good deal of heat. During the addition a white solid crystallized out, but this redissolved before the reaction was complete. After the addition anhydrous hydrogen chloride was passed into the solution until it was acid to Congo paper. On cooling β-aminoethylphenylsulfide hydrochloride crystallized out in white prisms. It melted in an anomalous fashion, half liquifying to a glass from 109-116°, and then remained unchanged till 162-163° where it melted to a clear liquid. Gabriel and Colman describe this compound as sintering from 110-120° and melting at 160-161°. That this is not due to solvation is indicated by the analytical data, since a purified sample showed the expected value in an ionic halogen determination.

Anal. Calcd. for $C_8H_{12}NSCl$ (189.5): Cl^-, 18.69%.

Found: Cl^-, 18.84%.

The product weighed 103.7 g., a yield of 78%.

β-Aminoethylphenylsulfide. - To prepare the free base 30.0 g. of the amine hydrochloride was dissolved in 100 ml. of absolute methanol. One equivalent of a solution of sodium methoxide in methanol was added, and the precipitated sodium chloride was filtered off. The solvent was removed in vacuo, the residual oil was taken up in isopropyl ether and filtered from an additional quantity of salt, the solvent was again removed, and the oil was distilled in vacuo. There was obtained 17.1 g., 71%, of a colorless liquid, b.p. 90-95°/0.18 mm. It could be reconverted to the amine hydrochloride indicating no decomposition had occurred on distillation.

d-N-Pantoyl-β-aminoethylphenylsulfide (SN 13592). - To the 17.1 g. of free base was added 16.0 g., a 10% excess, of l-pantolactone, and the mixture was maintained at 105° in an oil bath for five hours. It immediately formed a clear melt. After cooling the melt was taken up in 100 ml. of reagent benzene and extracted with two 25-ml. portions of 1% hydrochloric acid and then with successive portions of water till the aqueous extract gave no opalescence on basification. The benzene layer was dried with sodium sulfate and the benzene was removed by distillation in vacuo under nitrogen. To remove the last traces of solvent, the residual oil was kept at 100° and 0.05 mm. for two hours. This left 27.8 g., 88%, of a light green oil, very stiff and viscous at room temperature. It was completely insoluble in water, soluble with difficulty in isopropyl ether, and easily soluble in methanol and ethanol. The rotation of a

sample dissolved in methanol was determined and gave $[\alpha]_D^{23°} = 43.1°$ (0.752 g. in 25 ml. of methanol solution). Since the rotation of the original lactone was negative this confirms the fact that condensation had taken place. A sample of the oil was analysed and gave the following results. It lost 1% on drying to constant weight before analysis.

Anal. Calcd. for $C_{14}H_{21}NO_3S$ (283.4): C, 59.31%; H, 7.47%; N, 4.93%.

Found: C, 58.64%; H, 7.47%; N, 4.80%.

To check the structure of this compound a sample was hydrolysed. In 10 ml. of 0.5 N hydrochloric acid was placed 3.4 g. of the oil. It was insoluble, but went into solution in about twenty minutes when the suspension was refluxed. After two and one-half hours of heating, the solution was cooled and extracted thoroughly with ethyl acetate. On evaporation the ethyl acetate solution was found to contain 1.67 g. of l-pantolactone. The aqueous solution was concentrated to dryness in vacuo, the residual oil was taken up in ethanol and allowed to crystallize. There was obtained 1.20 g. of β-aminoethylphenylsulfide hydrochloride, melting as described previously at 105-110° and 155-158°.

The Preparation of d-N-Pantoyl-β-aminoethylphenylsulfone

The base required for this synthesis, β-aminoethylphenylsulfone, was prepared by Mr. Rapport by oxidation of β-aminoethylphenylsulfide hydrochloride with potassium permanganate. It has been previously described by Gabriel and Colman[22], who prepared it by the reaction of

β-bromoethylphthalimide with sodium benzenesulfinate, followed by hydrolysis of the phthalyl rest. The preparation of the free base and condensation were carried out much as recorded for the sulfide, except that the distillation of the free base was omitted when it was found that the final product was easy to purify.

d-N-Pantoyl-β-aminoethylphenylsulfone (SN 13594). - Nineteen and five-tenths grams of the hydrochloride of β-aminoethylphenylsulfone was dissolved in 80 ml. of anhydrous methanol, and a solution of 1.95 g. of sodium in methanol was added. The precipitate of sodium chloride was removed by filtration, and the methanol stripped off *in vacuo* under nitrogen. To the oily free base was added 15.0 g. of l-pantolactone, and the mixture was heated at 110° for five hours. (In a preliminary experiment the free base was distilled; β-aminoethylphenylsulfone came over at 149-152°/0.1 mm. with a good deal of loss due to decomposition.)

The melt was allowed to cool and taken up in 60 ml. of absolute ethanol. The solution was filtered from a small amount of sodium chloride, heated to boiling, and isopropyl ether added until crystallization appeared imminent. The volume was then about 350 ml. The first material to come out of solution was brown and gummy, and the mother liquors were decanted from this and cooled further. The product came out as gleaming white plates, m.p. 103-104.5° in a yield of 19.9 g., 72%. For purification this was placed in a Soxhlet extractor and extracted

with 400 ml. of isopropyl ether. The product was carried down into the boiler and crystallized out in colorless plates; about one gram of salt and brown gum was left in the thimble. The crystals weighed 18.5 g., and melted at 105.5-106.5°.

Anal. Calcd. for $C_{14}H_{21}O_5NS$ (315.4): C, 53.30%; H, 6.71%; N, 4.44%.
Found: C, 53.63%; H, 6.85%; N, 4.38%.

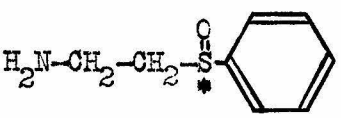
The optical rotation was determined in methanol. In 25 ml. of methanol solution 0.827 g. in a 20 cm. polarimeter tube gave an observed rotation of +2.62°. $[\alpha]_D^{27°} = +39.6°$.

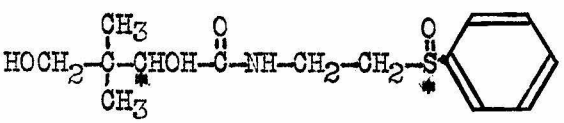
The Preparation of d-N-Pantoyl-β-aminoethylphenylsulfoxide

β-Aminoethylphenylsulfoxide hydrochloride which was required for this synthesis was prepared by oxidizing the corresponding sulfide hydrochloride with hydrogen peroxide, after the manner of Gadzer and Smiles[25]. Mr. Rapport in his first preparation of β-aminoethylphenylsulfone hydrochloride, by oxidation of this same sulfide hydrochloride with potassium permanganate, had used less than one equivalent of the oxidizing agent, had isolated this sulfoxide as a byproduct in a 12% yield, and had had it analysed; the compound obtained by hydrogen peroxide oxidation was identical with his analytical sample. The conversion of this salt to the free base went smoothly, but when an attempt was made to distill the free base an extensive alteration in the molecule occurred. A distillate was obtained which was insoluble in acid and analysed for an empirical formula of $C_{16}H_{14}OS_2$; this formula corresponds to two molecules of free base, less two molecules of ammonia and one

of water. Its character was not further investigated.

Purification of the final compound presented great difficulties. It will be noted that the free base contains an unsymmetrical sulfoxide grouping and should therefore be capable of resolution. In a condensation with an optically active lactone the product should be a mixture of two diastereoisomers. For this reason, as well as





because of the usual difficulties encountered with these compounds, the crystallization of the product was not accomplished. Solution of the product in an organic solvent and extraction with aqueous base and acid was prohibited by the unfavorable value of the distribution coefficient of the product between such solvent pairs. The final purification was effected by dissolving the reaction mixture in water, making the solution acid, and extracting the solution continuously with ethyl ether. As the lactone is quite soluble in ether, it was extracted rapidly. The rotation of successive extracts was determined at frequent intervals and when the value went from negative to positive it was assumed that extraction of lactone was essentially complete. A new ethyl ether extraction was then continued for twenty-four hours, removing the product into the ether pot. The product so obtained gave a satisfactory analysis and had a strong positve rotation,

comparable with that of the sulfide and sulfone. It should be noted that this procedure might produce some fractionation of the two diastereoisomers, so it is not correct to assume that they occurred in equal amounts in the material submitted for testing.

β-Aminoethylphenylsulfoxide hydrochloride. - In 90 ml. of water was dissolved 34.0 g. of β-aminoethylphenylsulfide hydrochloride, and 22.5 ml. of 30% hydrogen peroxide was added. The solution was warmed on a steam bath for four and one-half hours. The water was stripped off in vacuo; the residual yellow oil crystallized. The solid was dissolved in 250 ml. of boiling absolute ethanol, concentrated to 100 ml., and allowed to cool. There crystallized out 26.8 g., 73%, of white prisms, m.p. 156-158°. A mixed melting point of these crystals with an analytical sample of β-aminoethylphenylsulfoxide hydrochloride prepared by Mr. Rapport gave no depression.

Anal. Calcd. for $C_8H_{12}NOSCl$ (205.7): C, 46.70%; H, 5.88%; N, 6.81%.
Found: C, 46.82%; H, 5.99%; N, 6.84%.

d-N-Pantoyl-β-aminoethylphenylsulfoxide (SN 13593). - Twenty-six grams of the sulfoxide hydrochloride was dissolved in 100 ml. of methanol, and to the solution was added a solution of 2.70 g. of sodium in 100 ml. of methanol. The sodium chloride was removed by filtration and the methanol was distilled off in vacuo under nitrogen.

In a preliminary experiment the oily free base was distilled. It came over at 155-160°/0.15-0.25 mm. and crystallized in the receiver. The distillate after recrystallization from absolute ethanol melted at 59-60°.

Anal. Calcd. for $C_{16}H_{14}OS_2$ (286.4): C, 67.10%; H, 4.93%; N, 22.39%.

Found: C, 66.54%; H, 4.89%; N, 22.09%.

It was insoluble in dilute acid, although the hydrochloride of β-amino-ethylphenylsulfoxide was quite soluble in water.

To the viscous residue remaining after removal of the solvent from the free base was added 30.0 g. of l-pantolactone. The mixture was heated for eighteen hours at 100-105°. The melt was taken up in 200 ml. of water, brought to pH 2 with dilute hydrochloric acid, treated with Norite, and placed in a continuous liquid extractor. It was extracted with ethyl ether for varying periods, the extraction being interrupted from time to time to examine the material in the ether solution. This data is tabulated as follows.

Extract No.	Period of Extraction	Weight Extracted	Specific Rotation	Comments
1	1.5 hr.	4.3 g.	-39.5°	Crystalline, pure lactone
2	2.0 hr.	8.3 g.	-18.2°	Mainly lactone
3	1.5 hr.	4.1 g.	-10.7°	Mainly lactone
4	1.5 hr.	1.8 g.	- 3.7°	Lactone mostly gone
5	1.3 hr.	2.9 g.	+ 3.6°	Mainly product
6	1.5 hr.	1.5 g.	+ 8.8°	Mainly product
7	1.5 hr.	1.1 g.	+14.5°	May be pure product
8	23 hours	19.3 g.	+41.5°	Sent in for test

The product obtained in this eighth extraction was a pale yellow oil which was very viscous at room temperature. An analytical sample lost

4.25% of its weight on drying, probably representing solvent. It gave the following analysis.

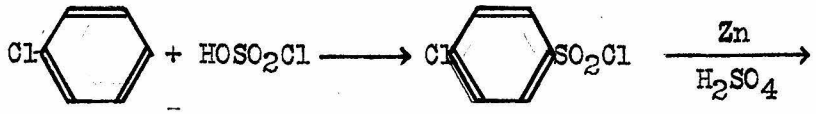
Anal. Calcd. for $C_{14}H_{21}NO_4S$ (299.4): C, 56.16%; H, 7.07%; N, 4.68%.
Found: N, 4.47%.

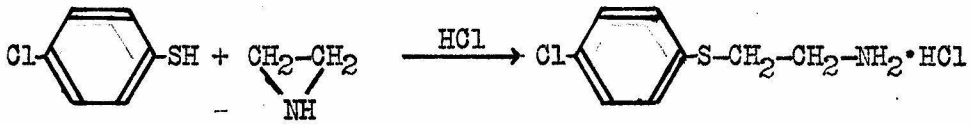
A sample prepared by this method in a preliminary experiment gave C, 55.86%; H, 7.71%; N, 4.44%. This is a yield of 51% based on the amine hydrochloride.

To check the structure of the product a sample of it was hydrolysed. In 25 ml. of water was dissolved 2.84 g. of the oil and 2 ml. of concentrated hydrochloric acid was added. The solution was refluxed for twenty hours, and then extracted with ethyl acetate; from this extract was recovered 1.10 g. of l-pantolactone. The aqueous solution was evaporated to dryness in vacuo, the crystalline residue was dissolved in absolute ethanol, and on cooling 1.12 g. of β-aminoethylphenylsulfoxide hydrochloride crystallized out. This melted at 156.5-158° and gave no melting point depression when mixed with a sample of the original sulfoxide hydrochloride used.

The Preparation of d-N-Pantoyl-β-aminoethyl-p-chlorophenylsulfide

The amine hydrochloride required for this synthesis was prepared by the series of reactions outlined below. The first two steps were carried out by Mr. Rapport, using the preparation of thiophenol[26] as a model.





The reaction between p-chlorothiophenol and ethylenimine proved to be very satisfactory. The amine hydrochloride showed the same peculiar type of melting point behavior exhibited by the unchlorinated analogue. In place of converting the amine hydrochloride to the free base under anhydrous conditions as in the previous cases, it was done, as described below, in aqueous solution. The condensation of this base with l-pantolactone and the isolation of the product were carried out as usual. The purity of the condensation product submitted for testing is shown by the analytical data on it and by its behavior in crystallizing after standing for some time.

β-Aminoethyl-p-chlorophenylsulfide hydrochloride. - In 300 ml. of absolute ethanol was dissolved 34.7 g. of p-chlorothiophenol. To the solution over a ten-minute period was added 11.2 g. of ethylenimine. An exothermic reaction occurred and the solution was cooled. After standing for ten minutes more, the solution was made acid to Congo paper with concentrated hydrochloric acid and placed in the cold room. The product crystallized out in thick white platelets and weighed 26.5 g.,

49%. By concentration of the mother liquors a second crop of 19.3 g., 36%, was obtained. This solid melted at a gel at 147-152°, remained unchanged on further heating, and finally melted at 231-233°. An analytical sample recrystallized from ethanol softened to a glass at 151-155° and melted at 230-232°.

Anal. Calcd. for $C_8H_{11}NSCl_2$ (224.2): C, 42.85%; H, 4.95%; N, 6.25%.
Found: C, 43.08%; H, 5.17%; N, 6.20.

d-N-Pantoyl-β-aminoethyl-p-chlorophenylsulfide (SN 14799). - A sample of amine hydrochloride was recrystallized twice from absolute ethanol and used in this preparation. Twelve and four-tenths grams of purified hydrochloride was dissolved in 100 ml. of water, and a solution of 3 g. of sodium hydroxide in 20 ml. of water was added. A white oil came out of the solution, which was taken up in 100 ml. of ethyl acetate. The acetate solution was dried with sodium sulfate and the solvent was removed in vacuo in a tared flask. The oily residue weighed 10.0 g. To it was added 10.0 g. of l-pantolactone, the air in the flask was swept out with nitrogen, and the flask was stoppered and placed in an oil bath at 90° for four hours.

The resulting oil was taken up in 200 ml. of reagent benzene, and the solution was thoroughly extracted with dilute sodium hydroxide, with dilute hydrochloric acid, and then with water. The benzene was removed in vacuo in a tared flask under nitrogen. To remove the last traces of solvent the residual oil was heated at 85° at 0.2 mm. for two hours. The yellow thick oil left weighed 13.0 g., a yield of 74%. This oil was sent in for biological testing.

A sample of the oil was submitted for analysis.

Anal. Calcd. for $C_{14}H_{20}NO_3SCl$ (317.8): C, 52.90%; H, 6.35%; N, 4.41%.

Found: C, 52.72%; H, 6.52%; N, 4.81%.

The optical rotation of a sample in methanol was determined; 0.767 g. in 25 ml. of methanol solution in a 10-cm. polarimeter tube gave an observed rotation of +1.28°. $[\alpha]_D^{20°} = +36.8°$.

After standing for several weeks this oil crystallized completely to give a light yellow solid, m.p. 56-58°.

The Preparation of d-N-Pantoyl-β-aminoethyl-p-chlorophenylsulfone

An attempt was made to oxidize β-aminoethyl-p-chlorophenylsulfide hydrochloride to the sulfone hydrochloride by the method employed by Madinaveitia et al.[17] in oxidizing phthalyl derivatives of such amines from sulfides to sulfones, namely oxidation by hydrogen peroxide in glacial acetic acid. The method worked, and the desired product was obtained and analysed, but the yield was small, and the product was quite impure. Therefore recourse was had to the method of oxidation employed by Rapport in the case of the unchlorinated sulfone, oxidation with potassium permanganate.

Converting the amine hydrochloride to the free base was done in the manner used in the previous preparation. When the amine hydrochloride dissolved in water was treated with sodium hydroxide at room temperature an oil was precipitated which crystallized on cooling. These crystals appeared to melt around 30°, but after thorough drying the melting point was raised to 70°. It seems likely that the base formed a low

melting, easily dissociated hydrate.

The condensation between the lactone and the free base probably was as satisfactory as in other cases, but the difficulties in working up the product led to a low yield. The material from the condensation was dissolved in ethyl acetate and washed with acid and base to remove unreacted materials. Apparently the final compound was soluble enough in water so that this produced significant losses. The unfortunate choice of calcium chloride as a drying agent for the ethyl acetate solution apparently led to loss of material, and caused the product to be contaminated with ash, so that a second purification was necessary, further lowering the yield. The product was submitted for testing in ethanol solution since attempts to cause the oil to crystallize had failed. No sooner was the solution in the mail then the analytical sample began to crystallize. Using seeds the product obtained in a preliminary condensation was caused to crystallize; in any future preparation use could be made of this to crystallize the whole product and doubtless greatly increase both the yield and the purity of the compound.

β-Aminoethyl-p-chlorophenylsulfone hydrochloride. - In a preliminary experiment 2.0 g. of β-eminoethyl-p-chlorophenylsulfide hydrochloride was oxidized with hydrogen peroxide by the method of Madinaveitia et al.[17] There were obtained 0.8 g. of impure crystalline material, m.p. 210-215°. An analytical sample after three recrystallization from ethanol melted at 218-219°.

Anal. Calcd. for $C_8H_{11}NO_2SCl_2$ (256.2): C, 37.52%; H, 4.33%; N, 5.47%.
Found: C, 37.53%; H, 4.23%; N, 5.72%.

Twenty grams of sulfide hydrochloride was dissolved in 160 ml. of water and cooled in an ice-salt bath, causing the amine hydrochloride to crystallize out and form a slurry. A solution of 18.8 g. of potassium permanganate and 9.7 ml. of concentrated hydrochloric acid in 500 ml. of water was added dropwise with stirring over a three-hour period, during which the temperature of the reaction mixture was maintained below 0°. The mixture was allowed to warm to room temperature and stand overnight.

The precipitated manganese dioxide was filtered off, and then extracted with 500 ml. of hot water. The filtrate and washings were concentrated in vacuo leaving a white crystalline residue. One hundred milliliters of ethanol was added, and removed in vacuo. The residue was extracted with 500 ml. of hot ethanol and filtered free of potassium chloride. A second extraction was performed, and the combined extracts were concentrated to 350 ml. On cooling, 14.9 g. of the sulfone hydrochloride crystallized out, which melted at 216-218°. Concentration of the mother liquors gave a second crop weighing 2.6 g. and melting below 195°. The yield of first crop material was 66%.

β-Aminoethyl-p-chlorophenylsulfone. - In a preliminary experiment 7.6 g. of sulfone hydrochloride was dissolved in 20 ml. of water, and a solution of 3.0 g. of sodium hydroxide in 20 ml. of water was added. A colorless oil came out. When the mixture was placed in the cold room the oil crystallized to form beautiful white plates. These were filtered off.

After air drying the plates appeared to melt at about 30°. On standing overnight in a desiccator over phosphoric anhydride the crystals changed into an oil which crystallized on scratching to give a white solid, m.p. 65-66°. On contact with water this solid changed to an oil which could be caused to crystallize again by cooling to 0°. A sample of the 66°-melting material was converted to the amine hydrochloride in quantitative yield with alcoholic hydrogen chloride, proving it was actually the free base. A sample was recrystallized from ethyl acetate; it melted at 79-80.5°.

d-N-Pantoyl-β-aminoethyl-p-chlorophenylsulfone (SN 13595). - One gram of this crystalline free amine was condensed with 0.65 g. of l-pantolactone at 95-100° for four hours. The resulting colorless oil was dissolved in ethyl acetate, and the solution was extracted with dilute acid. After removing the solvent the residual oil was placed in an icebox. Various attempts to cause it to crystallize failed, until the crystalline material mentioned below became available.

The large-scale preparation of the pantoylsulfone was carried out using 17.4 g. of twice recrystallized sulfone hydrochloride. This was dissolved in 100 ml. of water and 5.0 g. of sodium hydroxide in 20 ml. of water was added, both solutions being at 5°. A large crop of white platelets came out immediately on mixing the two solutions. These were filtered out and dried in a vacuum desiccator in the cold room. After the product was thoroughly dry it melted to a

glass at 69–72° and then changed little on heating further; apparently it contained some inorganic impurity. It weighed 13.0 g., 87%.

To this material in a flask was added 8.5 g. of l-pantolactone. The mixture was heated four and one-half hours at 95–100°. The resulting oil was dissolved in 100 ml. of ethyl acetate and extracted successively with three 30-ml. portions of cold 5% sodium hydroxide solution, three 30-ml. portions of cold 5% hydrochloric acid, and three 30-ml. portions of cold water. The ethyl acetate solution was finally dried over calcium chloride and sodium sulfate, and the ethyl acetate was removed *in vacuo* under nitrogen.

The residue was a white foam, which was freed of solvent as far as possible by heating it to 100° at 0.5 mm. for some time. The foam, which weighed about 10 g., became hard and easy to handle, although it was somewhat hygroscopic. An analytical sample of this was found to contain 10% ash; hence it was necessary to purify it further. To do this the foam was again taken up in ethyl acetate, and the solution was reextracted with two portions of water. This time the ethyl acetate solution was concentrated *in vacuo* without any drying. The residue was a clear yellow oil, which was freed of solvent by heating at 0.2 mm. and 100° for several hours. A sample of this was submitted for analysis. It lost 1.2% on drying to constant weight: the analytical figures on an undried sample follow.

Anal. Calcd. for $C_{14}H_{20}NO_5SCl$ (349.8): C, 48.06%; H, 5.76%; N, 4.00%.
Found: C, 48.67%; H, 6.15%; N, 3.89%.

This residue weighed 6.5 g. The optical rotation of a sample was determined; 67.7 mg. in 2.0 ml. of methanol solution gave a rotation of +1.23° in a 10-cm. polarimeter tube. $[\alpha]_D^{23°} = +36.3°$. Six and five-tenths grams of this oil was dissolved in redistilled ethanol, the volume of the solution being 19.5 ml. This solution was submitted for test.

On standing for several days the analytical sample of this oil crystallized. These crystals were used to seed the product of the preliminary run mentioned above, which immediately completely crystallized. This solid could be recrystallized from a large volume of isopropyl ether, from which it came out in rosettes of colorless crystals, thin, elongated platelets under the microscope. These melted at 103-105°. To recrystallize the main portion of this material it was placed in a Soxhlet extractor and treated with ethyl ether; it came out as nice crystals in the boiler.

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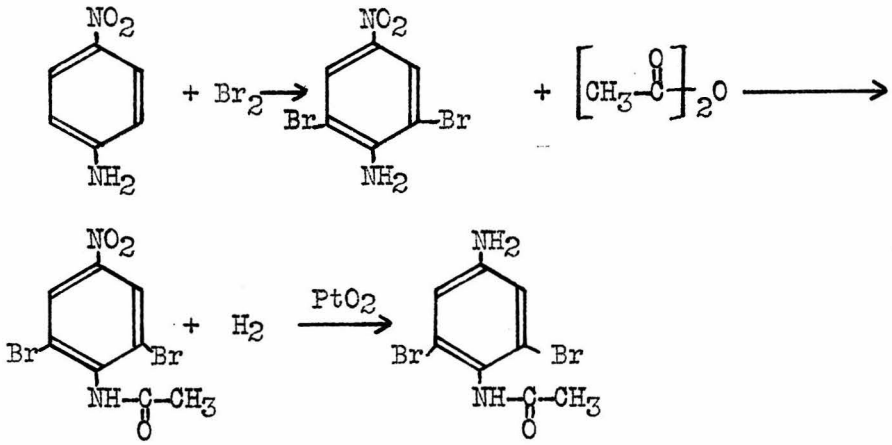
II. THE PREPARATION OF SOME SUBSTITUTED N^1-PHENYLSULFANILAMIDES

INTRODUCTION

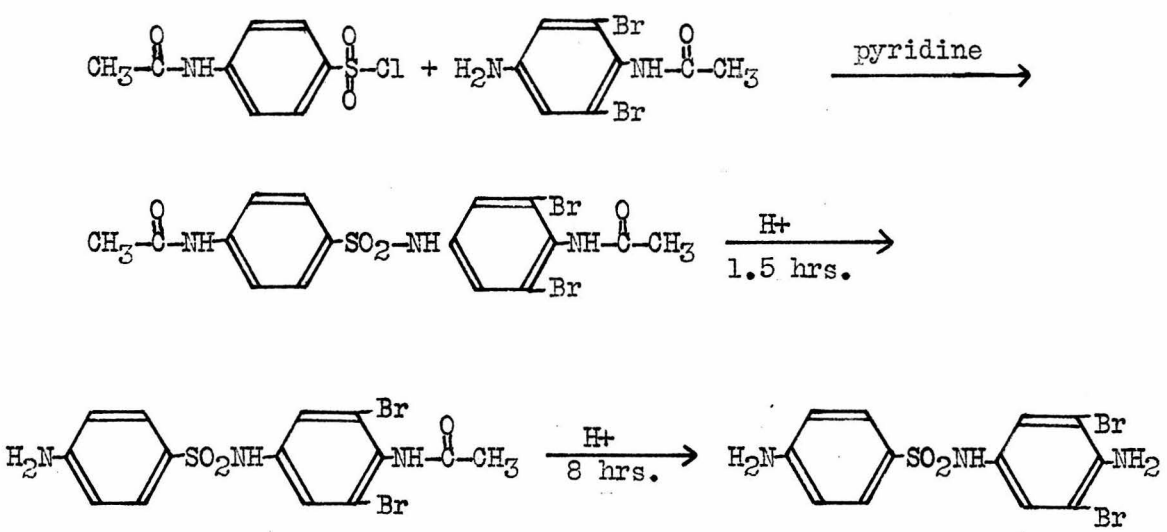
At one time considerable attention was directed towards the use of drugs of the sulfanilamide type as antimalarials, stimulated by the observation of Coggeshall and Maier[1] that rhesus monkeys infected with P. Knowlesi can be completely cured with sulfanilamide, and the work of Coggeshall, Maier, and Best[2] on the use of drugs of this sort in human malaria. During this period a number of N^1-phenylsulfanilamides were prepared on Dr. Koepfli's project.[3] In this thesis the syntheses of N^1-(3,5-dibromo-4-aminophenyl)-sulfanilamide and N^1-(3,5-dibromo-4-methylaminophenyl)-sulfanilamide are reported.

The procedure employed in both cases was that described specifically by Long and Burger[4] and followed the general plan which has been used in the large number of sulfanilamide syntheses which have been carried out in recent years. The appropriate amine was condensed with acetylsulfanilylchloride and the acetyl group was removed by hydrolysis, utilizing the much more rapid hydrolysis of carboxylic acid amides compared to sulfonamides.

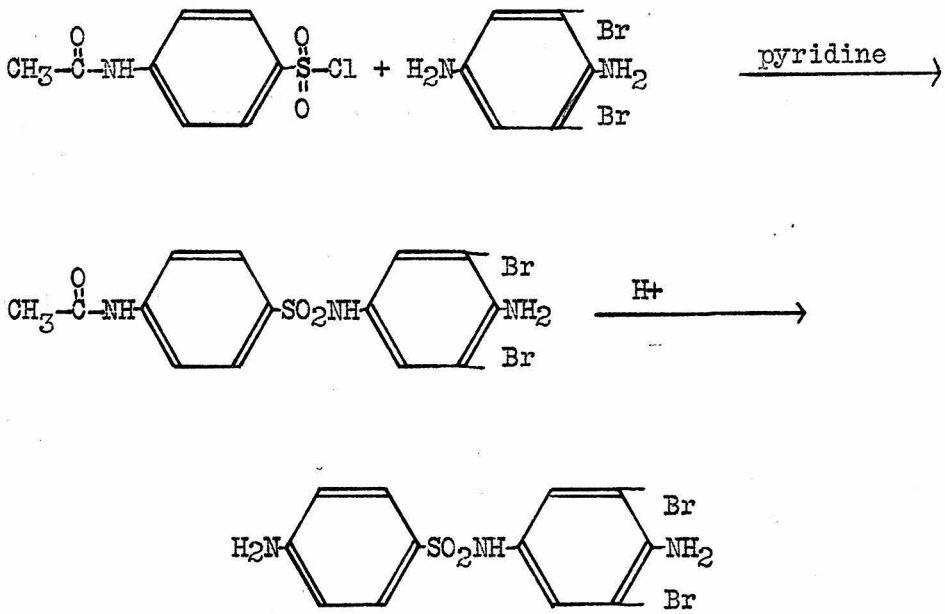
In the case of the first compound mentioned above the amine employed was 3,5-dibromo-4-acetaminoaniline which was prepared by Dr. Mead using the reactions shown. The amino group in the 4-position was acetylated to prevent it from condensing with the sulfonyl chloride.



After the condensation with acetylsulfanilylchloride, this blocking group was quite resistant to hydrolysis. Whereas the acetyl group in the N^4-position was removed by one and one-half hours of refluxing, it required about ten hours for the complete removal of the acetyl rest on the 4'-amino group. This was attributed to the presence of two bromine atoms ortho to the acetamino grouping.



It was suggested by Mr. John Maynard that this second acetyl group probably was not necessary since the steric effects hindering hydrolysis would destroy any tendency for the 4-amino group to condense with acetylsulfanilylchloride. To test this hypothesis he prepared a sulfonamide by condensing acetylsulfanilylchloride with 2,6-dibromo-p-phenylenediamine and, after hydrolysis, obtained a product in a better yield with less trouble which was identical with the final product of this synthesis.

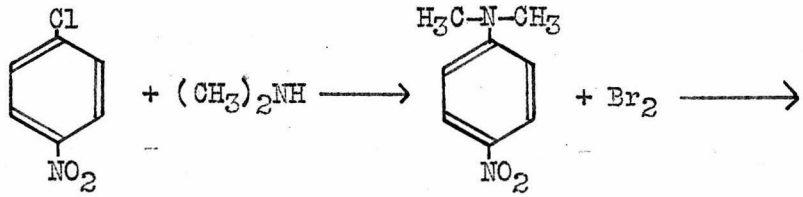


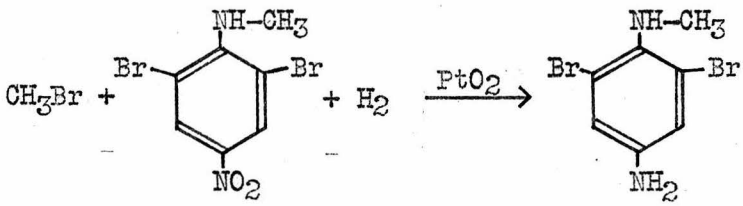
Maynard in his synthesis obtained a monoacetyl derivative of the final product in which the acetyl group must have been on the N^4-position. Therefore there can be no doubt that the monoacetyl derivative which was isolated as an intermediate during the hydrolysis described here was the compound with an acetamino group on the N^1-phenyl ring.

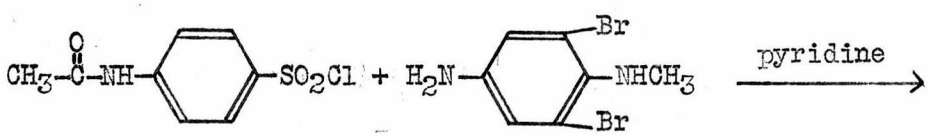
The preparation of N^1-(3,5-dibromo-4-methylaminophenyl)-sulfanilamide

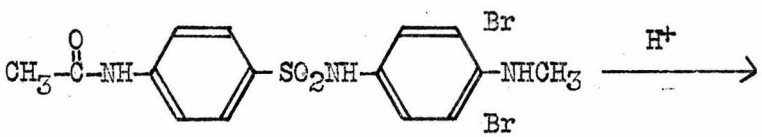
came about unexpectedly. An attempt was being made to prepare 4-nitro-2,6-dibromo-N,N-dimethylaniline to be used for the synthesis of N^1-(3,5-dibromo-4-dimethylaminophenyl)-sulfanilamide. The methylation of 4-nitro-2,6-dibromoaniline with methyl sulfate as described by Evans and Williams[5] for the methylation of p-nitroaniline failed. The formaldehyde-formic acid method described by Clark et al[6] for the methylation of 2,4,6-trisubstituted anilines would not work either. Therefore attention was directed to the bromination of p-nitro-N,N-dimethylaniline. This aniline was prepared by a sealed-tube reaction between p-nitrochlorobenzene and dimethylamine. It has been prepared previously by the methylation of p-nitroaniline and by the nitration of dimethylaniline, but this method which was suggested by Blanksma's[7] synthesis of p-nitro-N-methylaniline seemed to be simpler.

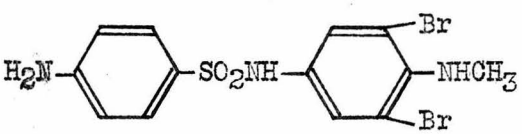
It was found, however, that when p-nitro-N,N-dimethylaniline was brominated a methyl group was split out giving a monomethyl compound. This is analogous to the result reported by Fries[8] during the bromination of dimethylaniline. The 4-nitro-2,6-dibromo-N-methylaniline obtained had been described by Blanksma[7] who obtained it on brominating p-nitro-N-methylaniline. The preparation of the sulfonanilamide from this compound thus unexpectedly obtained was undertaken since it would fill in the series of 4-amino compounds. In passing it might be of











interest to note that 4-nitro-2,6-dibromo-N,N-dimethylaniline was finally prepared by Mr. Rapport in a sealed-tube reaction between 4-nitro-2,6-dibromoiodobenzene and dimethylamine.

None of the compounds prepared and tested in this series showed any significant activity as antimalarials. In addition to the regular screening tests these compounds were tested for bacteriostatic action by Dr. Schmidt*. Their activity *in vitro* was equal to or greater than that of sulfathiazole against pneumococci, and less than that of sulfanilamide against Friedlander's bacillus. They were not as active *in vivo* against these organisms as sulfanilamide.

In view of the general picture of the mechanism of action of drugs of the sulfanilamide type now generally accepted, it was interesting to find that the bacteriostatic action of these compounds (as well as of the corresponding compounds with a dimethylamino group and a hydrogen in the 4-position) was not reversed by p-aminobenzoic acid. A similar observation has recently been made by Kaplan and Leubner[9]. These results indicate that certain sulfonamides have a bacteriostatic action of a different sort from that of sulfanilamide.

EXPERIMENTAL

N^4-acetyl-N^1-(3,5-dibromo-4-acetaminophenyl)-sulfanilamide. - Ten grams of 3,5-dibromo-4-acetaminoaniline was dissolved in 50 ml. of dry

*These tests will be described in the forthcoming monograph of the Survey on Antimalarial Drugs.

pyridine. Eleven and four-tenths grams of acetylsulfanilylchloride* was added slowly. The resulting solution stood several hours at room temperature and was then heated on a steam bath for two and one-half hours. The pyridine solution was poured onto a mixture of ice and hydrochloric acid and the precipitated solid worked free of pyridine. It was filtered, dried, and purified by being dissolved in 80 ml. of hot 2 N sodium carbonate and reprecipitated with hydrochloric acid. This yielded 13.3 g., 81%, of a crude product slightly contaminated with pyridine. A sample crystallized from ethanol and water melted at 236-238°.

N^1-(3,5-dibromo-4-acetaminophenyl)-sulfanilamide. - Thirteen and three-tenths grams of the crude diacetyl compound was dissolved in a mixture of 100 ml. of 95% ethanol and 30 ml. of concentrated hydrochloric acid. The solution was refluxed for one and one-half hours, filtered into 100 ml. of water, and neutralized with ammonia. On the addition of 300 ml. of water a white precipitate formed. The yield of this compound was 9.3 g., 76%. A sample after repeated recrystallization from aqueous ethanol melted at 210-213° and analyzed for a monoacetyl derivative of the desired product. Calculated for the free diamine $C_{12}H_{11}N_3O_2SBr_2$ (421.1): C, 34.2%; H, 2.63%. Calculated for a monoacetyl derivative $C_{14}H_{13}N_3O_3SBr_2$ (463.2): C, 36.3%; H, 2.83%. Found: C, 36.5%; H, 3.11%. N^4-acetyl-N^1-(3,5-dibromo-4-aminophenyl)-sulfanilamide prepared by Maynard melted at 232-233.5°.

*A generous supply of this material was given to us by Merck and Company.

N^1-(3,5-dibromo-4-aminophenyl)-sulfanilamide (SN 3864). - Eighteen and seven-tenths grams of this monoacetyl derivative was hydrolysed by the same procedure, eight hours of refluxing being employed. This produced 10.0 g., 60%, of a compound melting at 176-177° which was identical with the diamine obtained by Maynard.

Anal. Calcd. for $C_{12}H_{11}N_3O_2SBr_2$ (421.1): C, 34.2%; H, 2.63%; N, 9.8%.
Found: C, 34.5%; H, 2.69%; N, 10.3%.

p-Nitro-N,N-dimethylaniline. - Forty-four and eight-tenths grams of p-nitrochlorobenzene, 40 ml. of dimethylamine, and 200 ml. of 95% ethanol were mixed and sealed in four Carius tubes. The tubes were heated slowly over a five-hour period to 160° and held there for three hours. On cooling, the yellow product crystallized out. It was recrystallized from 1.5 l. of ethanol yielding 41.6 g., 88%, of p-nitro-N,N,-dimethylaniline melting at 163-166°.

2,6-Dibromo-4-nitro-N-methylaniline. - Eight and nine-tenths grams of p-nitro-N,N-dimethylaniline was suspended in 100 ml. of glacial acetic acid and a solution of 20 g. of bromine in 20 ml. of glacial acetic acid was added slowly while stirring vigorously. The mixture was stirred one-half hour at room temperature and one hour on a steam bath. A stream of air was passed through the suspension to remove excess bromine and the whole mixture was poured into 300 ml. of water. The precipitate was filtered and dried. It weighed 12.1 g. On crystallizing from ethanol, 5.0 g. of a product melting at 113-115° was obtained. Blanksma[9] gives

113° for the melting point of this compound. An analytical sample melting at 113.5-114.5° was prepared by crystallization from ethanol.

Anal. Calcd. for $C_7H_6O_2N_2Br_2$ (310.0): C, 27.1%; H, 1.95%; Br, 51.6%.
Found: C, 27.4%; H, 2.07%; Br, 51.9%.

On addition of water to the mother liquors, a second product was obtained, weighing 5.8 g. and melting at 59-61°. Its nature was not investigated. The yield of the desired product was thus 30%.

N^1-methyl-2,6-dibromo-p-phenylenediamine. - One gram of Raney nickel catalyst was hydrogenated for ten minutes at 45 lbs. hydrogen pressure. A solution of 10.3 g. of the nitro compound in 150 ml. of hot ethanol was added and the solution was hydrogenated for twenty minutes when the theoretical amount of hydrogen had been taken up. The catalyst was filtered off, the alcohol solution concentrated, and water added to opalescence, whereupon the amine crystallized out; 5.3 g., 54%, of crude amine being obtained. A sample recrystallized from a benzene-petroleum ether solution melted at 103-104°.

Anal. Calcd. for $C_7H_8N_2Br_2$ (280.0): C, 30.0%; H, 2.86%; N, 10.0%.
Found: C, 29.9%; H, 2.91%; N, 10.0%.

N^4-acetyl-N^1-(3,5-dibromo-4-methylaminophenyl)-sulfanilamide. - Eight and six-tenths grams of the amine was dissolved in 20 ml. of dry pyridine, and 8.3 g. of acetylsulfanilylchloride was added slowly. After one hour of warming on a steam bath the resulting solution was added to crushed ice. On working out pyridine a brown solid resulted. This was filtered, dried, and purified by dissolving in 30 ml. of 2 N sodium carbonate and reprecipitating with hydrochloric acid. This yielded 14.2 g., 92%, of a product melting at 217-220°. A sample recrystallized

from aqueous ethanol melted at 220-221.5°.

Anal. Calcd. for $C_{15}H_{15}N_3O_3SBr_2$ (477.2): C, 37.8%; H, 3.16%; N, 8.8%.
Found: C, 38.4%; H, 3.15%; N, 8.3%.

N^4-(3,5-dibromo-4-methylaminophenyl)-sulfanilamide (SN 3865). - Thirteen and eight-tenths grams of the acetyl compound was dissolved in a mixture of 200 ml. of ethanol and 60 ml. of concentrated hydrochloric acid. The solution was refluxed for one hour and poured into five volumes of water. The resulting solution was neutralized with ammonia, precipitating a brown solid. It was purified by solution in chloroform and precipitation with petroleum ether. The product weighed 10.0 g., 80%, and melted at 147-148.5° after recrystallization from a chloroform-ligroin mixture.

Anal. Calcd. for $C_{13}H_{13}N_3O_2SBr_2$ (435.2): C, 35.9%; H, 3.01%; N, 9.7%.
Found: C, 36.3%; H, 3.33%; N, 9.8%.

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4. L. Long and A. Burger, J. Am. Chem. Soc., 63, 1587 (1941).
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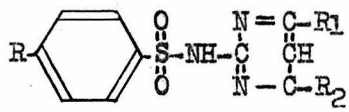
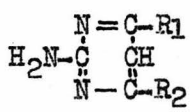
9. H. Kaplan and G. Leubner, J. Am. Chem. Soc., 67, 1076 (1945).

Table of Formulas for Section III

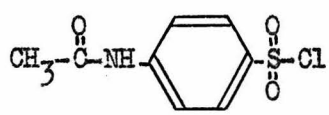


	R	R_1	R_2
I	H_2N-	Br-	H-
II	H_2N-	Br-	H_3C-
III	H_2N-	Br-	Br-
VII	AcNH-	C_2H_5O-	H_3C-
VIII	H_2N-	HO-	H_3C-
IX	AcNH-	HO-	H_3C-
X	AcNH-	Br-	H_3C-
XIV	AcNH-	CH_3O-	CH_3O-
XV	H_2N-	HO-	HO-

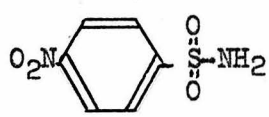


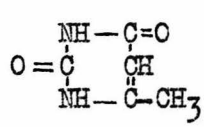
	R_1	R_2
VI	C_2H_5O-	CH_3-
XI	HO-	HO-
XII	Cl-	Cl-
XIII	CH_3O-	CH_3O-
XVI	Br-	H_3C-
XIX	Br-	Br-
XX	C_2H_5O-	H_3C-

	R	R_1	R_2
XVIII	O_2N-	Br-	H_3C-
XXI	O_2N-	C_2H_5O-	H_3C-
XXII	O_2N-	HO-	H_3C-
XXIII	O_2N-	Br-	H_3C-
XXIV	H_2N-	C_2H_5O-	H_3C-
XXIX	O_2N-	H_3C-	H-
XXX	O_2N-	H_3C-	H_3C-
XXXI	H-	C_2H_5O-	H-
XXXII	H-	HO-	H-

V 

XVII 

XXV 

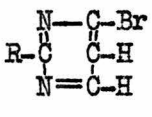
XXVI 

XXVII and XXVIII: Structure unknown.

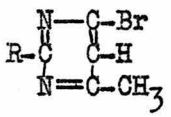
III. STUDIES ON SULFONAMIDOPYRIMIDINES

INTRODUCTION

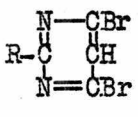
As mentioned in the preceding section of this thesis, at one time drugs of the sulfonamide type were thoroughly explored for antimalarial activity. Sulfadiazine, 2-(p-aminobenzenesulfonamido)-pyrimidine, was one of the most active and interesting members of this class. Therefore, a number of its derivatives were required for testing. To Dr. Koepfli's group was assigned the problem of preparing the brominated analogues of sulfadiazine I, II, III, and IV. At the same time Dr. Nathan L. Drake and his collaborators at the University of Maryland undertook the synthesis of the analogous compounds in which chlorine replaced bromine.



(I)



(II)



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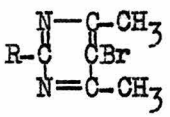

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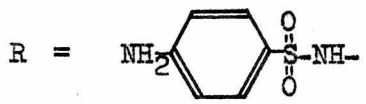
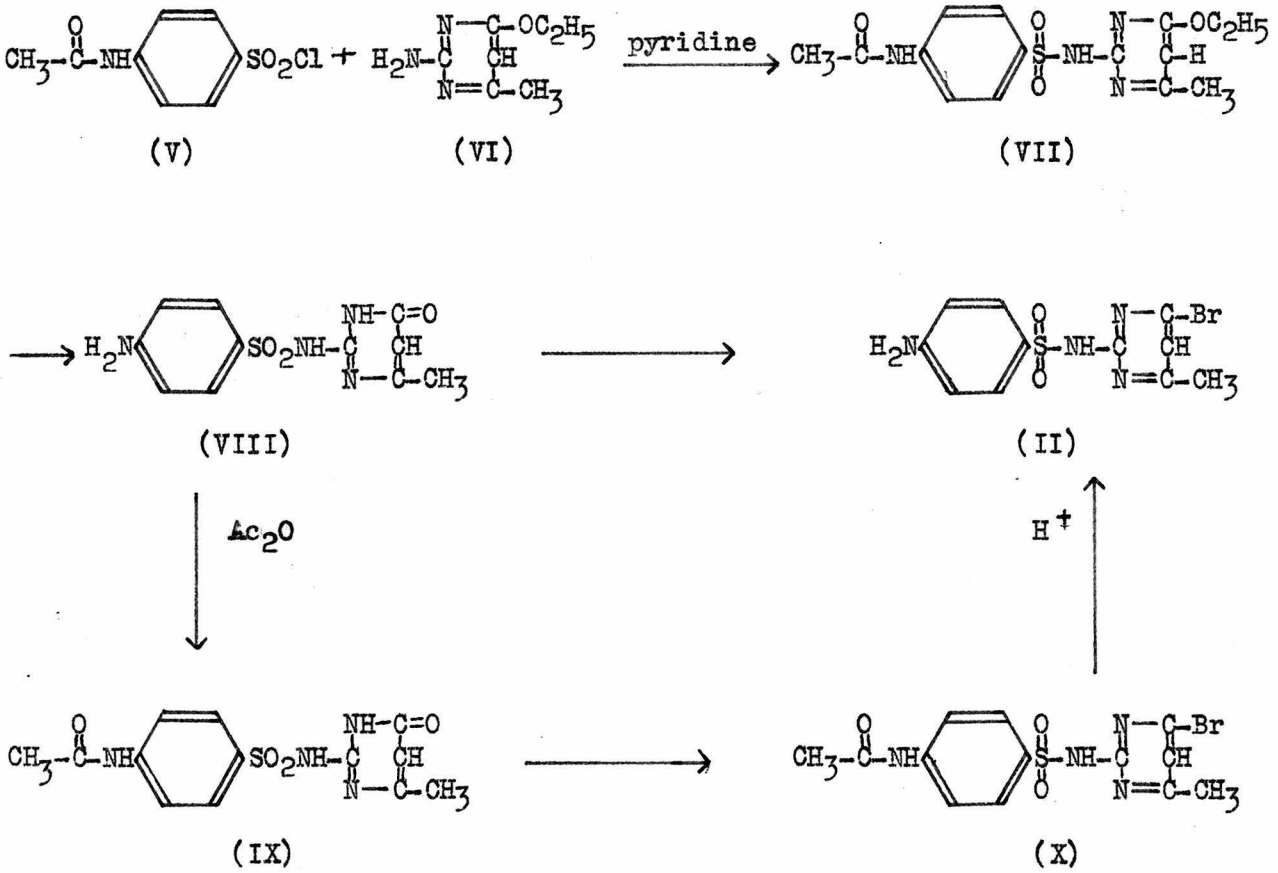


Compound IV had been described in the patent literature and was prepared again by Mr. J. T. Maynard, both by the condensation of 2-amino-4,6-dimethyl-5-bromopyrimidine with acetylsulfanilylchloride, followed by hydrolysis of the acetyl group and by bromination of 2-(p-aminobenzenesulfonamido)-4,6-dimethylpyrimidine. The syntheses of

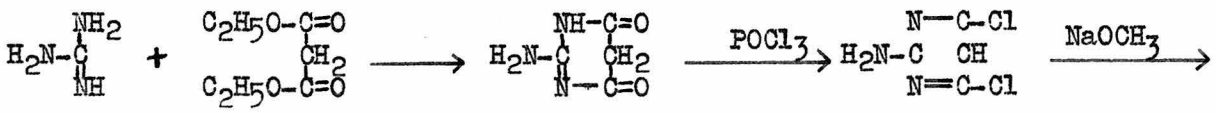
compounds I, II, and III were never carried out, although a great deal of time and effort was expended in the attempt. Dr. Drake and his group found it impossible to synthesize the analogous chloro compounds, so that until recently no compound with a p-aminobenzenesulfonamido group in the 2-position and a halogen in the 4- or 6-position of the pyrimidine ring had been prepared. In the last year Rose and Swain[1] have described the preparation of 2-(p-aminobenzenesulfonamido)-4-methyl-6-chloropyrimidine; a discussion of their paper and its relation to the work described here follows the experimental section.

This thesis describes the general problems encountered in attempting to prepare these compounds, and a portion of the experimental work done. In addition, a great deal of the pertinent experimental work was carried out by Mr. Rapport, Mr. Maynard, and Mr. Golding.

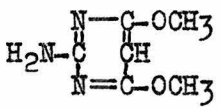
The first attempt to synthesize one of these compounds was made by Mr. Rapport. He prepared 2-(p-aminobenzenesulfonamido)-4-methyl-6-hydroxypyrimidine (VIII) and its acetylated derivative (IX) by the reactions shown, according to the directions of Sprague et al[2], and attempted to replace with bromine the 6-hydroxy group in either VIII or IX, using a variety of brominating agents and experimental conditions. He was unable to isolate either X or II in any of his reactions. Altogether he investigated about thirty different sets of conditions.



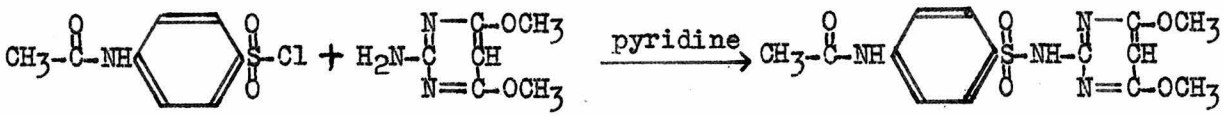
At this time Drake[3] reported that he was having no success in replacing these and similar hydroxyl groups with chlorine. It appeared that the sulfonamido group in the 2-position deactivated these hydroxyl groups too completely for this reaction to take place, and therefore this line of attack was abandoned. In the meantime, the preparation of 2-(p-acetaminobenzenesulfonamido)-4,6-dimethoxypyrimidine (XIV) had been carried out by the reactions shown; in view of Mr. Rapport's and Dr. Drake's results the hydrolysis of XIV and the bromination of XV was not attempted.



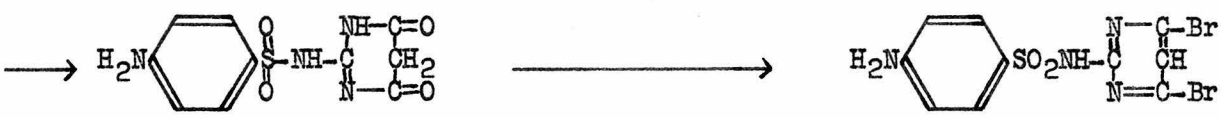
(XI) (XII)



(XIII)

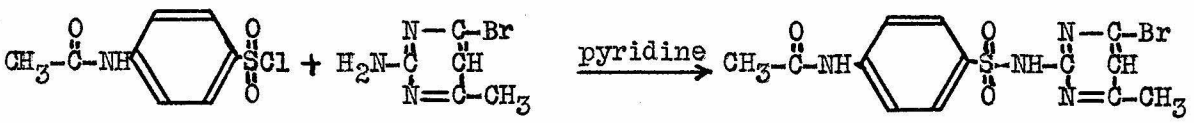


(V) (XIII) (XIV)



(XV) (III)

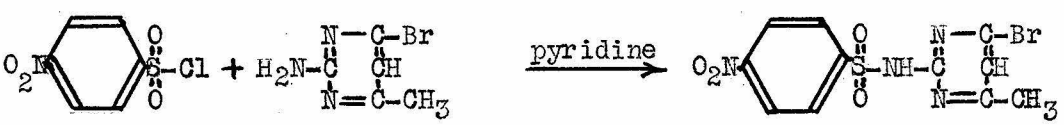
The next method which was investigated was the coupling of acetylsulfanilylchloride (V) with 2-amino-4-methyl-6-bromopyrimidine (XVI). This latter compound was prepared by Mr. D. R. V. Golding by treating 2-amino-4-methyl-6-hydroxypyrimidine with phosphorus oxybromide. Mr. Golding found it impossible to cause V and XVI to react to give X.



(V) (XVI) (X)

Dr. Drake[3] had failed to couple similar chloroaminopyrimidines with acetylsulfanilylchloride. Apparently, a halogen atom meta to the amino group deactivates the latter so strongly that it will not react with this sulfonyl chloride. Price et al[4] have also reported the failure of halogenated 2-aminopyrimidines to react with sulfonyl chlorides.

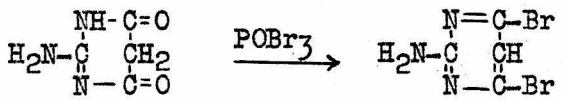
When this approach failed an attempt was made to employ the more reactive p-nitrobenzenesulfonylchloride (XVII). Several attempts to react this compound with 2-amino-4-halogenopyrimidines are described in the experimental section. Although in one instance a product was obtained in a reaction between XVI and XVII it did not have the properties expected for XVIII; it was probably a salt of p-nitrobenzene-sulfonic acid and 2-amino-4-methyl-6-bromopyrimidine.



(XVII) (XVI) (XVIII)

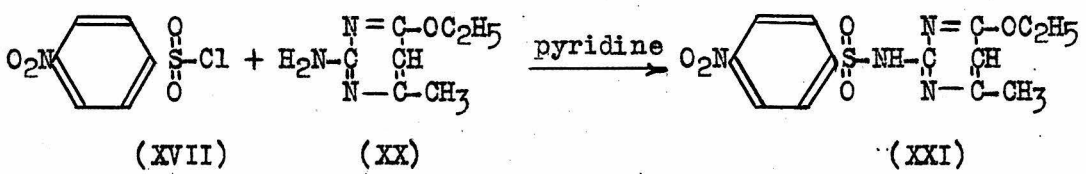
This approach was abandoned when it became apparent that using XVII instead of V did not lead to the desired product. During this phase

of the work the synthesis of 2-amino-4,6-dibromopyrimidine (XIX) was carried out by treating XI with phosphorus oxybromide.

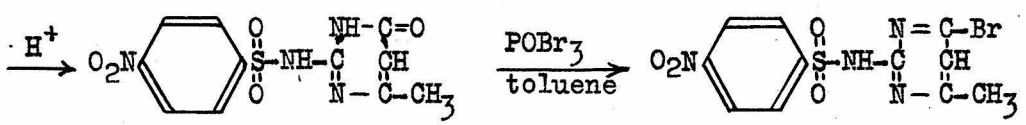


(XI) (XIX)

It was decided to try to brominate 2-(p-nitrobenzenesulfonamido)-4-methyl-6-hydroxypyrimidine (XXII), even though the corresponding p-acetamino compound IX and the p-amino compound VIII had resisted bromination. XXII was chosen since 2-amino-4-methyl-6-ethoxypyrimidine (XX) was the most readily available intermediate. The bromination of XXII was investigated using solvents in which phosphorus oxybromide was soluble. When it was shown that reaction took place readily in refluxing toluene, these specific conditions were tried on IX, but as before no reaction occurred.



(XVII) (XX) (XXI)



(XXII) (XXIII)

At this stage of the investigation, it seemed as though the major difficulty had been overcome since it was not anticipated that there would be too much trouble in reducing XXIII to II. However, when the reduction was attempted, this optimism proved to

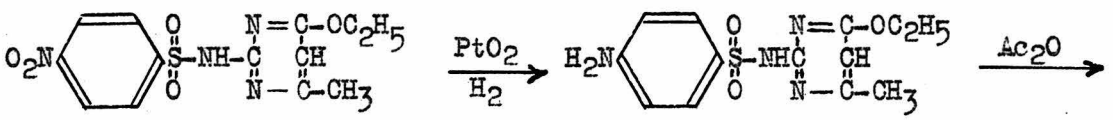
be unjustified. Although a large number of experiments were carried out, and a variety of methods of reduction were employed, in no case was it possible to isolate a product which could be identified as II. Among the methods of reduction investigated were reduction with iron powder and hydrochloric acid in ethanol, iron powder in acetic acid, zinc dust in pyridine, sodium hydrosulfite, ferrous sulfate and ammonia (hot), stannous chloride in hydrochloric acid, and catalytic reduction using either Raney nickel or platinum oxide (Adams') as catalysts.

The products which were obtained varied from reaction to reaction. They were all, however, white amorphous powders, which decomposed to black, charred material at high temperatures without melting. They were soluble in dilute base and in some cases were partly soluble in dilute acid. They could not be crystallized from any solvents and could be purified for analysis only by reprecipitation from solution in base. When analyses were carried out on such reprecipitated samples, the data obtained was meaningless. Several typical reductions are described in the experimental section; other similar cases could be given but would add nothing to the picture. A possible explanation of these results is advanced in the discussion following the experimental section, based in part on the nature of the chloro analogue of II prepared by Rose and Swain[1].

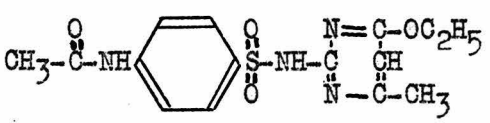
At this point it was decided that enough time had been spent trying to synthesize these compounds. In any case the emphasis in the antimalarial program had shifted from the sulfonamides to other classes of drugs.

Accordingly, the investigation was dropped. For that reason the work which is described in this section of the thesis is somewhat incomplete, and there are a number of unexplained experimental observations.

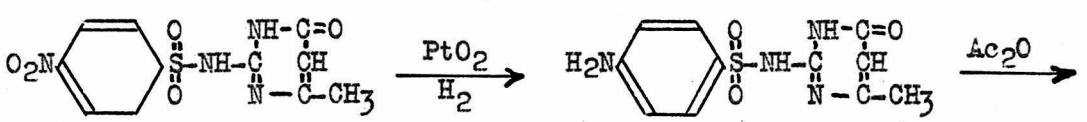
During the course of the investigation of the reduction of XXIII, several experiments were carried out to determine if XXIII was actually 2-(p-nitrobenzenesulfonamido)-4-methyl-6-bromopyrimidine. First, the structures of XXI and XXII were checked by converting them to the corresponding amino and acetamino compounds of Sprague et al[2] (see equations below). Samples of these compounds were available from Mr. Rapport for mixed melting points. As a final check, XXII was hydrolysed to give p-nitrobenzenesulfonamide (XXV) and 4-methyluracil (XXVI) proving the structure conclusively.



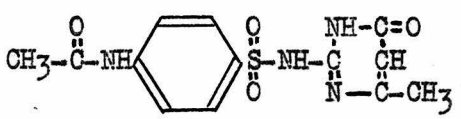
(XXI) (XXIV)



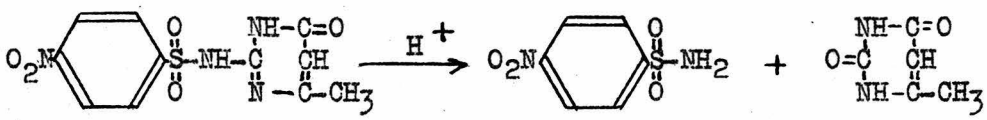
(VII)



(XXII) (VIII)

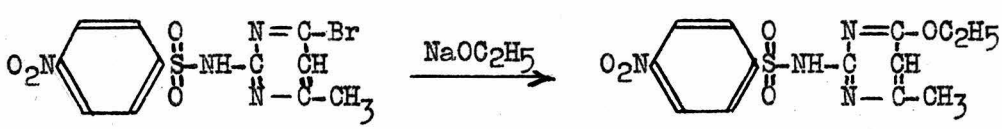


(IX)



(XXII) (XXV) (XXVI)

An attempt was made to prove the structure of XXIII directly. That the compound contained bromine in organic combination was proved by dissolving it in dilute base and reprecipitating it unchanged with carbon dioxide, treatment which would decompose a hydrobromide. XXIII was refluxed with sodium ethoxide in the hope that it would be converted to XXI; apparently some deep-seated de-

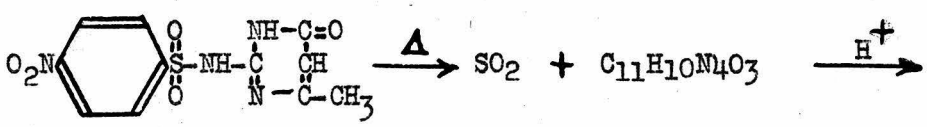


(XXIII) (XXI)

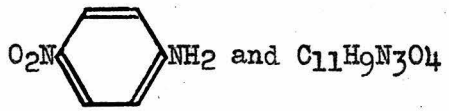
composition occurred and no crystalline product could be isolated. When XXIII was hydrolysed using acid, a large yield of p-nitrobenzene-sulfonamide (XXV) was obtained, confirming the sulfonamide structure of XXIII, but no identifiable fraction from the pyrimidine ring could be isolated. Therefore the evidence for the structure of this bromo compound is all indirect.

While working on the structures of these compounds, an interesting reaction was discovered. XXII decomposed at about 215° with formation of a new crystalline compound; this was first noted in a melting point tube. Therefore, an analytical sample of XXII was heated until this reaction had been completed and was then analyzed. The analytical data

indicated that the decomposition involved the splitting of sulfur dioxide from the molecule, yielding a new compound XXVII. This behavior seemed to warrant further investigation in connection with the difficulties encountered in reducing XXIII. Accordingly, some hydrolytic experiments were carried out on XXVII. As indicated by the equation XXVII gave two products, p-nitroaniline, and a compound XXVIII, formed from XXVII by replacing a nitrogen and a hydrogen with oxygen. XXVIII could also be obtained from



(XXII) (XXVII)



(XXVIII)

XXVII by treatment with nitrous acid. A number of other sulfonamides were tested to see if they would undergo similar transformations; of those tested only XXI and 2-(p-nitrobenzenesulfonamido)-4-hydroxypyrimidine would do so.

Several other new sulfonamides were prepared in the course of this work. 2-(p-Nitrobenzenesulfonamido)-4-methylpyrimidine (XXIX) was prepared because the product of the iron-hydrochloric acid reduction of XXIII gave analytical data which would fit its empirical formula, although the properties of the reduction product were not compatible

with such a structure. It seemed that the difficulties in the reduction of XXIII might have been due to the presence of an active methyl group in the molecule; to test this 2-(p-nitrobenzenesulfonamido)-4,6-dimethylpyrimidine (XXX) was prepared, and its reduction to 2-(p-aminobenzenesulfonamido)-4,6-dimethylpyrimidine (sulfametazine) was carried out. 2-Benzenesulfonamido-4-ethoxypyrimidine (XXXI) and 2-benzenesulfonamido-4-hydroxypyrimidine (XXXII) were prepared to see whether they could be made to lose sulfur dioxide and furnish analogues of XXII. As noted above they did not do so.

EXPERIMENTAL

2-Amino-4,6-dihydroxypyrimidine (XI). - This compound was prepared according to the directions of Michael[5], except that the quantity of sodium employed was doubled. In 300 ml. of ethanol was dissolved 16 g. of sodium, and the resulting solution was added to a solution of 31.8 g. of guanidine hydrochloride in 100 ml. of warm ethanol. After cooling, sodium chloride was filtered off, and 53.9 g. of diethyl malonate was added to the filtrate. In about ten minutes a white crystalline precipitate began to form.

After standing overnight the crystals were separated, washed with ether, and air-dried. They were dissolved in 450 ml. of water, and XI was precipitated by bringing the solution to neutrality with hydrochloric acid. The precipitate weighed 38.3 g., 91%, and was pure enough for the next step.

2-Amino-4,6-dichloropyrimidine (XII). - This was prepared by treating crude XI with phosphorus oxychloride as described by Buttner[6]. The product was purified by crystallization from ethanol instead of by vacuum sublimation.

2-Amino-4,6-dimethoxypyrimidine (XIII). - This compound was readily prepared by reacting XII with sodium methoxide as described by Fisher and Johnson[7].

2-(p-Acetaminobenzenesulfonamido)-4,6-dimethoxypyrimidine (XIV). - Two grams of 2-amino-4,6-dimethoxypyrimidine was dissolved in 6 ml. of reagent pyridine and 3.65 g. of acetylsulfanilylchloride was added in small portions. The resulting solution was allowed to stand overnight. When it was poured into 75 ml. of water, an oil precipitated which solidifed when the pyridine was worked out. It weighed 3.9 g. after drying over phosphoric anhydride in vacuo. An analytical sample crystallized from absolute ethanol consisted of thin, colorless bars, m.p. 244-245°.

Anal. Calcd. for $C_{14}H_{16}N_4O_5S$ (352.4): C, 47.71%; H, 4.58%; N, 15.90%.
Found: C, 47.95%; H, 4.72%; N, 15.74%.

2-Amino-4,6-dibromopyrimidine (XIX). - In a test tube were placed 1.27 g. of 2-amino-4,6-dihydroxypyrimidine (XI) and 5.00 g. of phosphorus oxybromide*. On heating the tube to 135°, a vigorous reaction was initiated, involving the evolution of hydrogen bromide.

*Prepared by Mr. D. R. V. Golding.

When the reaction subsided, the mass solidified. Cracked ice was added, the solid was triturated and filtered. A sample was purified by crystallization from ethanol, sublimation in vacuo, and recrystallization from ethanol. It consisted of bundles of flat rectangular plates and melted at 189.5-192.5°.

Anal. Calcd. for $C_4H_3N_3Br_2$ (252.9): C, 19.00%; H, 1.19%.

Found; C, 19.11%; H, 1.42%.

In a second reaction a 32% yield of partially purified material was obtained upon crystallizing the crude reaction product from ethanol. This is the highest yield which was been obtained in this type of reaction and makes this compound the most readily available of the 2-amino-4-bromopyrimidines.

p-Nitrobenzenesulfonylchloride (XVII)[8]. - This acid chloride was prepared by the method given in the references cited. Rose and Swain[1] have recently described a simpler preparation.

p-Nitrobenzenesulfonylchloride (XVII) and 2-amino-4-halogenated pyrimidines. - An attempt was made to carry out the condensation of XVII with 2-amino-4-methyl-6-chloropyrimidine by heating the reactants in pyridine. Upon pouring the solution onto ice, the solid which precipitated contained no sulfonamide as indicated by the absence of any base-soluble fraction in it. A similar result was obtained when 2-amino-4-methyl-6-bromopyridine* was used. When the reaction of XVII with two mols of 2-amino-4-bromopyrimidine** in benzene was investigated, the amine

*Prepared by Mr. D. R. V. Golding.
**Prepared by Mr. J. T. Maynard using Golding's method.

was recovered unchanged. A similar result was found when toluene was substituted for benzene as the solvent.

In one such reaction, a product was obtained which at first was thought to be the desired sulfonamide. In a mixture of 5 ml. of acetone and 5 ml. of water was placed 463 mg. of sodium bicarbonate and 860 mg. of 2-amino-4-methyl-6-bromopyrimidine. To the suspension was added in small portions 1220 mg. of p-nitrobenzenesulfonylchloride. The sulfonyl chloride dissolved slowly with the evolution of carbon dioxide. This solution was added to 20 ml. of water, and the acetone was allowed to evaporate. A white crystalline solid came out consisting of rectangular plates weighing 920 mg. These melted at 168-170°. The product was insoluble in dilute sodium hydroxide solution and in dilute hydrochloric acid, but was soluble in concentrated hydrochloric acid. An analytical sample melted at 171-172.5°.

Anal. Calcd. for $C_{11}H_9N_4SBr$ (373.2): C, 35.40%; H, 2.43%; N, 15.02%; Br, 21.14%.

for $C_{11}H_9N_4SBr \cdot H_2O$ (391.2): C, 33.78%; H, 2.83%; N, 14.33%; Br, 20.27%.

Found: C, 34.21% (34.95%); H, 2.83% (2.77%); N, 13.43%; Br, 20.27%.

The first empirical formula is that of 2-(p-nitrobenzenesulfonamido)-4-methyl-6-bromopyrimidine. Although the analytical results fit the second formula more closely, the data is not reliable enough to permit a decision on this basis. This product might have been a salt of p-nitrobenzenesulfonic acid and 2-amino-4-methyl-6-bromopyrimidine. That it was not

XVIII is apparent from its insolubility in dilute base and the fact that it was entirely different from the compound which was obtained in the bromination of XXII, to which the structure XVIII was assigned. When this solid was shaken with dilute base, filtered, and dried, the melting behavior of the residue suggested that it was partly 2-amino-4-methyl-6-bromopyrimidine; this is what one would expect from a salt.

2-Amino-4-methyl-6-ethoxypyrimidine (XX). - This compound was prepared following the directions of Sprague et al[2].

2-(p-Nitrobenzenesulfonamido)-4-methyl-6-ethoxypyrimidine (XXI). - In 90 ml. of pyridine was placed 29.0 g. of XX, and 51.8 g. of XVII was added over a five-minute period. After one-half an hour at room temperature the solution was heated one hour on a steam bath and then poured into 600 ml. of water. The solution was made acid, the product was filtered off, and reprecipitated from sodium hydroxide solution. The solid weighed 57.2 g., 90%, and decomposed at 211° after sintering from 195°. It could be crystallized from ethanol, giving light grey or yellow crystals, decomposing at 209-211°. An analytical sample after several crystallizations consisted of colorless, flat, elongated platelets which decomposed at 207.5-208.5° after darkening for several degrees.

Anal. Calcd. for $C_{13}H_{14}N_4O_5S$ (338.3): C, 46.14%; H, 4.17%; N, 16.56%.
Found: C, 46.27%; H, 4.23%; N, 16.17%.

2-(p-Nitrobenzenesulfonamido)-4-methyl-6-hydroxypyrimidine (XXII). - Fifteen grams of crude XXI was suspended in 75 ml. of concentrated hydrochloric acid and 250 ml. of ethanol. On refluxing the solid dissolved, but a crystalline precipitate soon appeared. After boiling two hours the mixture was cooled, and the product removed by filtration. It weighed 11.2 g., 81%, and showed a most peculiar behavior on melting. It decomposed with evolution of some gas at 218-221°, forming a gel or glass, which crystallized and finally melted at 285-287°.

After recrystallization from acetic acid the product weighed 10.0 g., 73%.

Anal. Calcd. for $C_{11}H_{10}N_4O_5S$ (310.3): C, 42.57%; H, 3.25%; N, 18.06%.

Found; C, 42.31%; H, 3.71%; N, 17.94%.

On subsequent runs it was found that the temperature of the first decomposition tended to vary within the range 210-230°, although the reaction was always complete within a two- or three-degree range.

2-(p-Nitrobenzenesulfonamido)-4-methyl-6-bromopyrimidine (XXIII). - The bromination of XXII was carried out by suspending it in a refluxing toluene solution of phosphorus oxybromide. This was a much cleaner method than the neat reaction employed in the preparation of the aminobromopyrimidines. Using benzene as a solvent gave no reaction. Xylene appeared to be satisfactory, although some darkening of the suspended mass occurred; this reaction mixture was never worked up due to the success of the reaction in toluene. Acetic acid was tried as a solvent, since it dissolved both reactants, but no reaction took place. When an attempt was made to use acetic anhydride as a solvent it was found that it reacted with phosphorus oxybromide alone.

Fifteen grams of phosphorus oxybromide was dissolved in 60 ml. of dry toluene, and 6.5 g. of XXII was added. The suspension was refluxed for seven hours. After cooling, ice was added, themixture was shaken to decompose unreacted phosphorus oxybromide, and the product was recovered by filtration. It melted at 190-198°(dec.) and weighed 7.5 g. It was crystallized from a mixture of 250 ml. of ethanol and 200 ml. of water to give 6.1 g., 78%, of material melting at 210.5-212.5° (dec.). An analytical sample consisted of thin white bars.

Anal. Calcd. for $C_{11}H_9N_4O_4SBr$ (373.2): C, 35.40%; H, 2.43%; N, 15.02%; Br, 21.42%.

Found: C, 35.11%; H, 2.39%; N, 15.12%; Br, 24.3 %.

Attempted reduction of 2-(p-nitrobenzenesulfonamido)-4-methyl-6-bromopyrimidine(XXIII). - In a flask fitted with a reflux condenser and a stirrer were placed 3.73 g. of XXIII, 10 g. of iron powder, 30 ml. of ethanol, and 0.2 ml. of concentrated hydrochloric acid. The mixture was heated and stirred for seven hours. The cooled solution was neutralized with sodium hydroxide, and filtered at the boiling point. The residue was washed with hot ethanol. To the filtrate was added 400 ml. of water, causing a white powder to come out. This weighed 380 mg., and was soluble in dilute base, but not in dilute acid. On heating to 300° in a melting-point tube, it turned into a black clinker, but did not melt. By concentrating the filtrate 450 mg. of addition material was obtained. A portion of the product was dissolved in hot ethanol and precipitated with water. After a sodium fusion the material gave a strong positive test for sulfur and a negative test for halogen.

Anal. Calcd. for $C_{11}H_{10}N_4O_4S$ (292.3): C, 44.90%; H, 3.43%; N, 19.05%.
for $C_{11}H_{12}N_4O_3S$ (280.3): C, 47.12%; H, 4.32%; N, 19.98%.
Found; C, 46.51%; H, 4.35%; N, 19.51%.

The former of these formulas fits 2-(p-nitrobenzenesulfonamido)-4-methylpyrimidine (XXIX), but as shown below this product in no way resembled XXIX.

A similar reduction was carried out using iron dust in glacial acetic acid as the reducing agent. The product obtained from this reaction was very similar in properties to that just described. (Found: C, 48.33%; H, 4.55%; N, 7.03%.) It is difficult to explain the low nitrogen found for this compound.

A number of other chemical methods of reduction were investigated; they gave compounds which were like those described above. Due to difficulties in obtaining the products from the reaction mixtures they were not as useful. Among the reagents tried were sodium hydrosulfite, stannous chloride in hydrochloric acid, zinc in pyridine, and ferrous sulfate and ammonia. This type of reduction was abandoned, since the production of halogen-free products was not desired.

In the Burgess-Parr apparatus 1.50 g. of XXIII dissolved in ethanol was reduced catalytically, using Raney nickel. When the absorption of hydrogen had ceased, the catalyst was filtered off and the solvent was removed in vacuo. The residual white powder was triturated with water and filtered. The filtrate was acidic and gave a precipitate with silver nitrate. The residue was a solid which did not melt below 280°, at which temperature it was charred and black. After solution in ethanol and precipitation with water, the material gave strong positive tests for sulfur and halogen.

Anal. Calcd. for $C_{11}H_{11}N_4O_2SBr$ (343.2): C, 38.48%; H, 3.23%; N, 16.33%; Br, 23.28%.

Found; C, 45.90%; H, 4.64%; N, 16.84%; Br, 9.82%.

The low bromine excludes the possibility of this product being II.

A number of similar reductions using both platinum oxide and Rainey nickel were carried out. The properties of the products varied in a confusing fashion. They were usually at least partially soluble in dilute hydrochloric acid, although in one instance this was not so. This solubility in dilute hydrochloric acid could be destroyed easily. In several cases solution of the product in dilute base and precipitation with dilute acid destroyed it. In one case an acid-soluble product was extracted with refluxing ethanol for one hour; at the end of this period it has lost its acid solubility and contained no halogen. An analysis was obtained on this material. (Found: C, 46.42%; H, 4.75%; N, 12.85%.)

An attempt was made to hydrolyse one of these reduction products. The acid-soluble material from a catalytic hydrogenation was dissolved in 25% hydrochloric acid. On warming the solution, a white precipitate came out. After three hours it was removed by filtration and analysed, after reprecipitation from base. (Found: C, 46.69%; H, 4.55%; N, 7.61%.) Halogen was absent from the molecule.

Although a good deal of effort was directed towards finding some way of purifying these materials, the only thing which could be done was to reprecipitate them from solution in base. This would not be expected to provide any fractionation of the materials. They were not soluble in any of the common organic solvents except ethanol; from ethanol they came out as amorphous powders only on adding water. When no method of reduction

could be found which would give a product having the analytical data or the expected properties of II, the synthesis was regretfully abandoned.

Reduction of 2-(p-nitrobenzenesulfonamido)-4-methyl-6-ethoxypyrimidine (XXI). - At 45-1b. hydrogen pressure 3.4 g. of XXI was shaken with 0.5 g. of Raney nickel catalyst. When no reduction took place, the catalyst was filtered off, the solution was heated to boiling, fresh catalyst was added, and the reduction resumed. When the absorption of hydrogen ceased, the catalyst was filtered off, the solvent concentrated *in vacuo*, and the residue precipitated from base. This solid melted to a thick red gel at 105-113° with evolution of a gas. The gel finally liquified at 149-152°. According to Sprague *et al*[2] 2-(p-aminobenzenesulfonamido)-4-methyl-6-ethoxypyrimidine (XXIV) melts at 151-152° and forms a hydrate from dilute ethanol melting at 104-105°. Since it was difficult to obtain this material anhydrous, a sample of it was converted to the N-acetyl derivative, VII, using acetic anhydride and sulfuric acid. This melted at 242-246° and gave no depression of melting point when mixed with a sample of VII prepared by Mr. Rapport.

Reduction of 2-(p-nitrobenzensulfonamido)-4-methyl-6-hydroxypyrimidine (XXII). - This compound was reduced catalytically in the same fashion. The product after reprecipitation from acid melted at 252.5-254°. A sample of 2-(p-aminobenzenesulfonamido)-4-methyl-6-hydroxypyrimidine (VIII), prepared by Mr. Rapport, melted at 252-254°; a mixed melting point of the two gave no depression. As a final check

the N-acetyl drivative, IX, was prepared. It melted at 270-272°, checking the value reported for IX by Sprague et al[2].

Hydrolysis of 2-(p-nitrobenzenesulfonamido)-4-methyl-6-hydroxypyrimidine (XXII). - In 10 ml. of concentrated hydrochloric acid was placed 2.5 g. of XXII and 13 ml. of water. Upon refluxing the solid slowly dissolved. After twenty-four hours the solution was cooled. A white crystalline precipitate formed which melted at 170-175° and weighed 1.80 g. On heating with ethanol a portion of the solid would not dissolve; this was filtered off and saved. On cooling the ethanol, yellow crystals came out, which were shown to be p-nitrobenzenesulfonamide (XXV) by comparison with a sample of XXV prepared from p-nitrobenzenesulfonylchloride and ammonia. The alcohol insoluble material melted at 315-319°. It was soluble in water and acetic acid, and insoluble in ethanol. The material (200 mg.) recrystallized from ethanol-water consisted of bundles of white bars, m.p. 316-138° (dec.).

Anal. Calcd. for $C_5H_6N_2O_2$ (126.1): C, 47.63%; H, 4.80%; N, 22.22%.
Found: C, 46.95%; H, 4.76%; N, 21.91%.

This is the empirical formula for 4-methyluracil (XXVI). According to Beilstein[9] the melting point of 4-methyluracil is 320° (dec.). In a second run of this hydrolysis the same two products were obtained, XXV in 84% yield and XXVI in 62% yield.

Hydrolysis of 2-(p-nitrobenzenesulfonamido)-4-methyl-6-bromopyrimidine (XXIII). - Two grams of XXIII was refluxed with 20 ml. of 25% hydrochloric acid for forty hours. On cooling the resulting solution, some brown crystals appeared, which weighed 600 mg., and melted at 179-181°. These were recrystallized several times from

ethanol and then melted at 182-183°. (Found: C, 35.63%; H, 3.03%; N, 13.92%.) This fits the calculated values for p-nitrobenzenesulfonamide (XXV)m $C_6H_6N_2O_4S$ (202.2): C, 35.64%; H, 2.99%; N, 13.86%. The identity of this product with XXV with confirmed by its failure to lower the melting point of an authentic sample of XXV. This was a yield of 55% of the crude sulfonamide.

Decomposition of 2-(p-nitrobenzenesulfonamido)-4-methyl-6-hydroxypyrimidine (XXII). - The analytical sample of XXII was heated to 225° in an oil bath until the evolution of gas had ceased, when it had a decomposition point of 284-287°. It was then submitted for analysis.

Anal. Calcd. for $C_{11}H_{10}N_4O_3$ (246.2): C, 53.65%; H, 4.09%; N, 22.75%.
Found: C, 53.65%; H, 4.05%; N, 22.32%.

In a large-scale experiment 7.3 g. of XXII was heated in a flask in an oil bath at 230°. The escaping gas was identified as sulfur dioxide both by its odor and by its reducing action on a solution of sodium dichromate. After the evolution of gas was complete the residue was taken up in dilute hydrochloric acid, filtered from a small amount of unreacted material, and reprecipitated with sodium bicarbonate. The product (XXVII) weighed 4.3 g., 74%, and melted at 271-275° (dec.).

A more elegant method of carrying out this decomposition was heating with strong base. Ten grams of XXII was dissolved in 50 ml. of hot 6 N sodium hydroxide. Upon heating the solution with steam a precipitate began to form; after several minutes the reaction was complete and the product was filtered off. It weighed 8.1 g., a 100% yield.

It was of interest to note that apparently this reaction could be brought about under acidic conditions. In studying the hydrolysis of XXI one reaction was carried out using concentrated hydrochloric acid instead of the alcoholic hydrochloric acid generally employed. The main product obtained was p-nitrobenzenesulfonamide, in a yield of 47%. In addition XXII was isolated in a 10% yield and XXVII in a 5% yield; presumably the latter arose from XXII under the influence of the acid.

Hydrolysis of XXVII. - In a mixture of 10 ml. of hydrochloric acid and 13 ml. of water was placed 2.5 g. of XXVII. This gave a clear solution. After fifteen hours of refluxing some solid had come out. This was removed by filtration and weighed 750 mg. The filtrate and washings were neutralized with dilute ammonia which precipitated a fine white solid, weighing 1.0 g. and melting from 143-147°. This latter product was dissolved in ethanol, some insoluble material was filtered off, and, after concentrating the solution, the material was precipitated by adding water. The yellow needles obtained melted at 147-149.5°. This agrees with the literature value for the melting point of p-nitroaniline. As a check the N-acetyl derivative of the product was prepared and compared with N-acetyl-p-nitroaniline prepared from commercial p-nitroaniline.

The solid which had crystallized out of the hydrolysis mixture was next investigated. It melted at 265-285° and was soluble in base but not in acid. This is just the reverse of the solubilities of XXVII. It was recrystallized from acetic acid, giving white crystals melting at 340-342°.

Anal. Calcd. for $C_{11}H_9N_3O_4$ (247.2): C, 53.44%; H, 3.67%; N, 17.00%.
Found: C, 53.84%; H, 3.96%; N, 17.44%.

This is the empirical formula of a degradation product of XXVII in which an -NH group has been replaced by an -O. It should be noted that this means that this material (XXVIII) is not formed along with p-nitroaniline; it must either be formed by a separate line of hydrolysis, or else it must be an intermediate in the formation of p-nitroaniline. No pyrimidine fraction corresponding to the p-nitroaniline produced was found. The yield of crude p-nitroaniline obtained was 71%; that of XXVIII 30%.

A hydrolysis of XXVII was carried out, in which 1.5 g. of the material was refluxed with 50 ml. of water and 2 ml. of sulfuric acid for twenty-four hours. There was obtained a 74% yield of crude XXVIII and a 25% yield of p-nitroaniline.

Action of nitrous acid on XXVII. - In a solution of 60 ml. of water and 10 ml. of acetic acid heated to 60° was placed 1.5 g. of XXVII. Then 1.66 g. of sodium nitrite was added in small portions. After a few minutes a solid began to form. After one hour at 60° the mixture was cooled and the solid filtered off. This solid was suspended in water, the water was made basic, and the undissolved solid was filtered off. On acidification the filtrate precipitated a second product. In this way the product was separated into two fractions, 400 mg. of base soluble material melting at 305-310° (dec.) and 610 mg. of material insoluble in base. The base soluble fraction after purification proved to be XXVIII. The base insoluble material was a light yellow solid, which turned a vivid red and evolved a gas at about 145°, leaving a semi-solid mass. This may have been a nitroso compound; when it could not be crystallized, no further work was done with it.

2-(p-Nitrobenzenesulfonamido)-4-methylpyrimidine (XXIX). - 2-Amino-4-methylpyrimidine was prepared by the reduction of 2-amino-4-methyl-6-chloropyrimidine with hydrogen in the presence of palladium catalyst, as described in the literature[10]. In 3 ml. of pyridine 980 mg. of this amine was reacted with 2240 mg. of p-nitrobenzenesulfonylchloride. After standing at room temperature for twelve hours, the product was isolated in the usual fashion. The product was only partially soluble in 40 ml. of 3% sodium hydroxide solution; the insoluble portion was filtered off, and the desired compound was precipitated with dilute acid. After crystallization from acetic acid it melted at 253-256° (dec.) and consisted of bundles of thin bars.

Anal. Calcd. for $C_{11}H_{10}N_4O_4S$ (292.2): C, 44.90%; H, 3.43%; N, 19.05%.
Found: C, 45.21%; H, 3.89%; N, 19.24%.

This bore no resemblance to the product of the reduction of the bromo compound with iron powder.

2-(p-Nitrobenzenesulfonamido)-4,6-dimethylpyrimidine (XXX). - Two grams of 2-amino-4,6-dimethylpyrimidine* and 3.75 g. of p-nitrobenzenesulfonylchloride were dissolved in 6 ml. of reagent pyridine. The solution stood 15 minutes at 40° and 15 minutes at 70°. It was poured into water, acidified, and the product filtered off. After reprecipitation from base and recrystallization from ethanol, the product was obtained as a mat of colorless needles, m.p. 215.5-216°.

Anal. Calcd. for $C_{12}H_{12}N_4O_4S$ (308.3): C, 46.74%; H, 3.92%; N, 18.17%.
Found: C, 46.82%; H, 4.10%; N, 17.84%.

*Prepared by Mr. J. T. Maynard.

This material was reduced catalytically, using the conditions described previously for the reduction of such nitro groups. The product was a solvate, losing solvent at 127° and melting at 181-183°. Rose and Swain[1] describe 2-(p-aminobenzenesulfonamido)-4,6-dimethylpyrimidine as melting at 178-180° after initial melting and resolidification at 127-130°. (Calculated for $C_{12}H_{14}N_4O_2S$ (278.3): C, 51.79%; H, 5.07%. Found after drying to constant weight: C, 51.58%; H, 4.98%.)

2-Benzenesulfonamido-4-ethoxypyrimidine (XXXI). - Ten grams of 2-amino-4-ethoxypyrimidine* and 14.1 g. of benzenesulfonylchloride were coupled in 20 ml. of Merck pyridine. The solution was heated forty-five minutes on the steam bath; the product was then isolated as usual. After recrystallization from acetic acid it weighed 11.5 g., 57%, and melted at 233-238°. An analytical sample from acetic acid consisted of hexagonal platelets and melted at 236-238°.

Anal. Calcd. for $C_{12}H_{13}N_3O_3S$ (279.3): C, 51.63%; H, 4.70%; N, 15.05%.
Found: C, 51.42%; H, 5.07%; N, 14.81%.

2-Benzenesulfonamido-4-hydroxypyrimidine (XXXII). - This was prepared by hydrolysing XXXI under the conditions used to convert XXI to XXII. After two and one-half hours of refluxing 3.0 g. of XXXI gave 2.2 g. of crude XXXII, melting at 189-191.5°. A thrice-recrystallized sample melted at 200-201° and consisted of beautiful needles.

*Prepared by Mr. J. T. Maynard.

Anal. Calcd. for $C_{10}H_9N_3O_3S$ (251.3): C, 47.79%; H, 3.62%; N, 16.71%.
Found: C, 48.64%; H, 3.67%; N, 15.55%.

These values indicate that the product contains some unhydrolysed material.

Attempted decompositions of sulfonamidopyrimidines. - In view of the production of the decomposition product XXVII when XXII was melted or warmed in strong base, it was of interest to see if similar decomposition products could be isolated from any other sulfonamidopyrimidines. Accordingly, several of these were tested both by heating and by warming with base. Only two compounds wefe found which underwent similar reactions. As might be expected, 2-(p-nitrobenzenesulfonamido)-4-hydroxypyrimidine gave a product analogous to XXVII; this was done by Mr. J. T. Maynard. The other compound which was found to lose sulfur dioxide was 2-(p-nitrobenzenesulfonamido)-4-methyl-6-ethoxypyrimidine (XXI). In this case the reaction was not quantitative. The reaction had to be carried out by heating a basic solution of the sulfonamide; when XXI was melted it decomposed with the evolution of sulfur dioxide (detected by odor), but the decomposition was quite extensive, and the product was black.

Three grams of XXI was dissolved in 0.4 N sodium hydroxide. Attempts to use stronger basic solutions resulted in the precipitation of the sodium salt of XXI. This solution was refluxed for two hours. It turned red and precipitated a solid which, when it was filtered off and dried, weighed 820 mg. and melted at 110-120°. The filtrate was acidified. A strong odor of sulfur dioxide was noted, and a large

precipitate of unchanged XXI came down. The alteration product was crystallized from aqueous ethanol. It melted at 146-147° and consisted of yellow needles.

Anal. Calcd. for $C_{13}H_{14}N_4O_3$ (274.2): C, 56.92%; H, 5.14%; N, 20.46%.
Found: C, 56.63%; H, 5.48%; N, 20.02%.

Among the compounds which failed to show this reaction were those lacking the p-nitro group, including VII, VIII, IX, XXXI, and XXXII. The two p-nitro compounds, XXIX and XXX, which did not have an oxygen substituent on the pyrimidine ring also failed to react.

DISCUSSION

Some light may be thrown on the difficulties which were encountered in reducing 2-(p-nitrobenzenesulfonamido)-4-methyl-6-bromopyrimidine by referring to the paper by Rose and Swain[1] already noted. These workers prepared 2-(p-nitrobenzenesulfonamido)-4-methyl-6-hydroxypyrimidine (XXII) from 2-p-nitrobenzenesulfonamidoguanidine and ethyl acetoacetate. This was converted to the corresponding chloro compound by refluxing the sulfonamide with phosphorus oxychloride, giving a product melting at 203-205°. By heating this with ammonia in phenol at 140° they prepared 2-(p-nitrobenzenesulfonamido)-4-methyl-6-aminopyrimidine, which was then reduced to a diamine using iron and a little hydrochloric acid. When this method of reduction was applied to the chloro compound it failed; using ferrous sulfate and ammonia at room temperature, however, the reduction succeeded. The chloro analogue of II thus obtained could be purified by crystallization from ethanol, but when it was dissolved

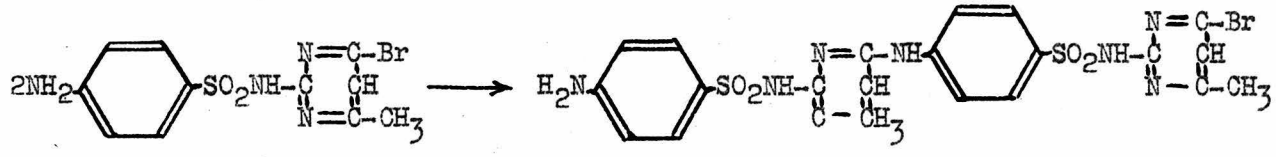
in dilute hydrochloric acid and heated for a short time it threw out a white solid, presumably due to intermolecular condensation.

On the basis of this work and of the results described in the experimental section, it would appear that upon reduction of XXIII the desired product, II, may have been formed, but that it was unstable and polymerized to form high molecular weight products. Presumably when the reduction was carried out using vigorous conditions, such as those described using iron and hydrochloric acid, this polymerization took place during the reaction, and the product which was isolated was a polymer, insoluble in acid. When the milder catalytic conditions were employed, it seems likely that at least part of the material obtained was the desired product, but that it was destroyed in attempting to isolate and purify it.

The fact that the product from the iron and hydrochloric acid reduction is free from halogen, while the acid soluble product from the catalytic hydrogenation contained halogen, suggests that this polymerization is concerned with the elimination of halogen; this in turn might explain why the product of catalytic hydrogenation was so unstable. Rose's compound apparently was stable in boiling ethanol, since it was crystallized from this solvent, whereas the reduction products described above were profoundly altered by heating with ethanol. If the polymerization reaction postulated was concerned with the alkylating action of the bromopyrimidine, this could be accounted for in terms of the greater activity of bromine compared to chlorine in such reactions. That the halogen atom is concerned in this behavior is confirmed by the

ease with which the other nitro sulfonamides were reduced as described in the experimental section, although this does not prove that it was the halogen acting as an alkylating agent which was responsible for the anomalous behavior.

The loss in solubility in dilute acid which accompanies this change suggests strongly that the amino group is concerned. It is tempting to conjecture that the polymerization might involve the addition of the bromopyrimidine to the amino group, giving the type of structure pictured. Unfortunately there is not enough evidence to decide such a question.



It would be desirable to attempt to convert XXIII to 2-(p-nitrobenzene-sulfonamido)-4-methyl-6-aminopyrimidine, using the procedure of Rose and Swain[1], as a check on the structure of XXIII. It would also be of interest to see if XXIII could be reduced to II using the mild chemical reducing method employed by Rose and Swain.

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IV. THE SYNTHESIS OF SOME QUINOLINEMETHANOLS RELATED TO QUININE

INTRODUCTION

In 1938 Ainley and King[1] reported the preparation of two quinolinemethanols which had certain structural similarities to quinine without possessing the complexity which had up to that time prevented the synthesis of the natural alkaloid. They gave directions for the synthesis of α-(2-piperidyl)-4-quinolinemethanol (I) and its 6-methoxy derivative (II). These compounds and the general scheme of synthesis are shown in Figures I and II. In 1921 Ruzicka[2] had made an attempt to prepare the former of these but had been forced to abandon it due to the low yields encountered in the initial Claisen condensation between ethyl cinchoninate and ethyl ω-benzamidocaproate, the instability of the intermediate ketones, and the difficulties of the final reduction. The increase in yield resulting from replacing sodium ethoxide with sodamide as a condensing agent enabled Ainley and King to overcome the first difficulty, the other two were alleviated by altering the experimental methods.

These two compounds together with several of their N-alkylated derivatives were tested for quinine-like activity against avian malaria. The 6-methoxy compound gave positive results and the authors claimed that this constituted the first instance of activity in a synthetic compound patterned after quinine. In spite of this promising conclusion nothing more appears to have been done by these workers on compounds of this type.

In the course of the present program it was desired to retest these

No.	R_1	R_2	R_3	R_4	Q
I	H-	H-	H-	H-	0.08
II	H-	CH_3O-	H-	H-	——
III	ϕ-	H-	H-	H-	3
IV	H-	H-	Cl-	H-	0.3
V	ϕ-	H-	Cl-	H-	10
VI	C_6H_{11}-	H-	H-	H-	0.15
VII	ϕ-	Cl-	Cl-	Cl-	——
VIII	ClC_6H_5-	Cl-	H-	Cl-	40

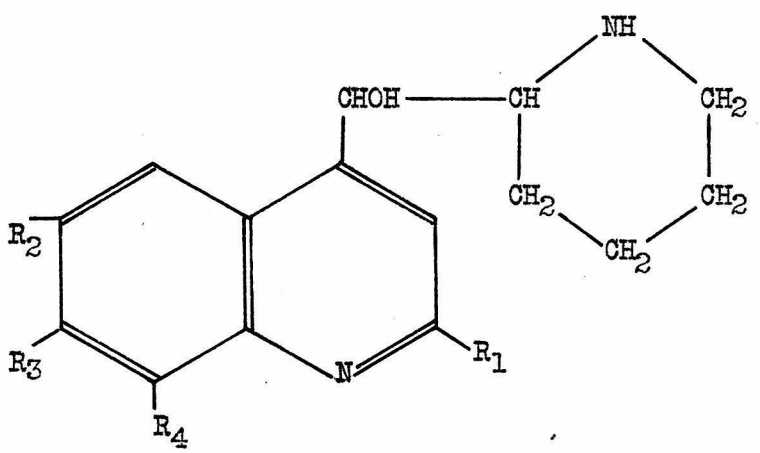


Figure I. The Structures and Activities of Ainley-King Type Compounds.

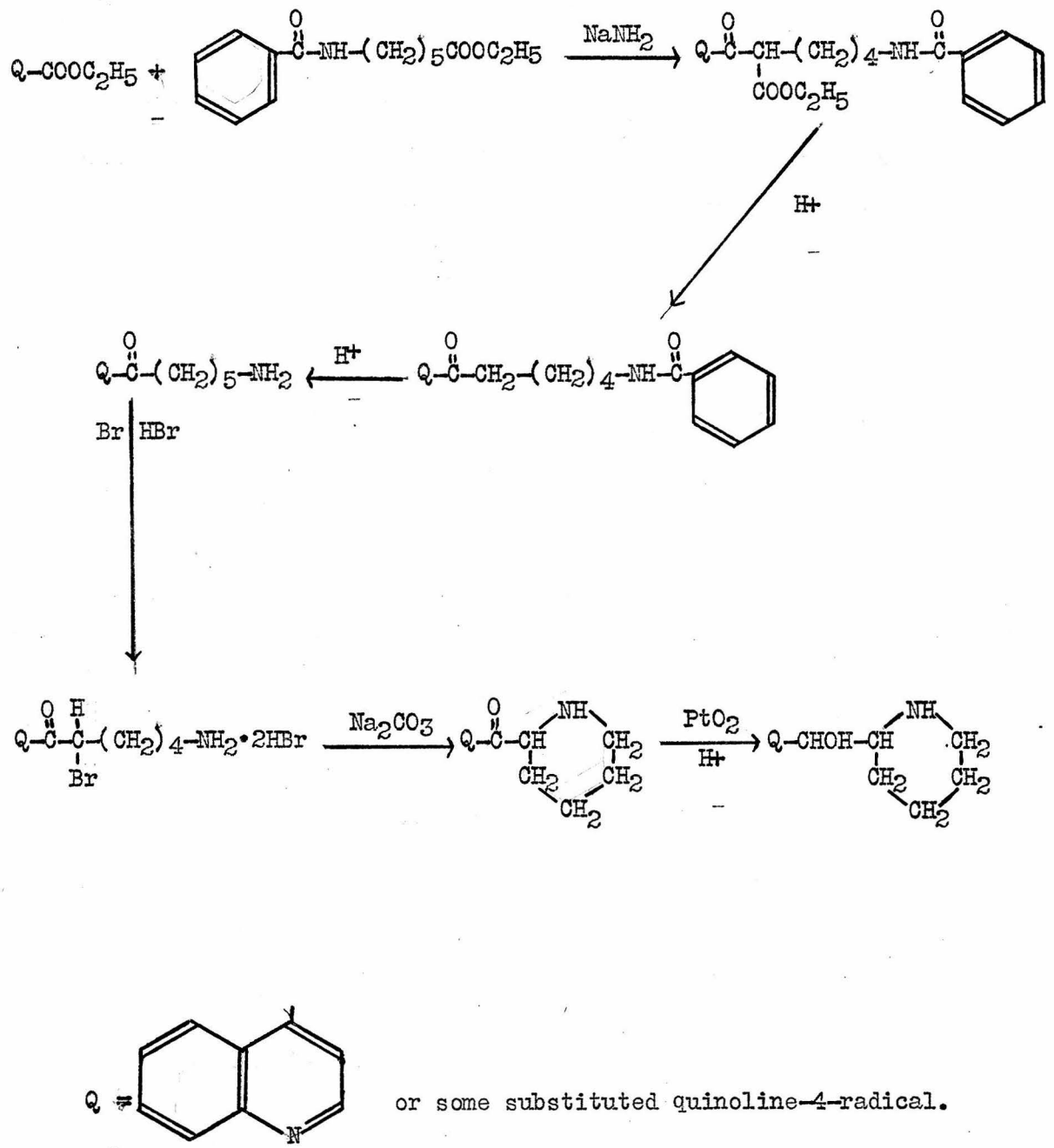


Figure II. The Synthesis of Ainley-King Type Compounds

compounds using the screening methods developed by the Survey on Antimalarial Drugs. Therefore the Ainley-King synthesis was thoroughly reinvestigated by Sargent[3]. He introduced a number of improvements in technique which simplified the manipulations involved and increased the yield. The main difference was the demonstration that it was possible to carry out the synthesis without isolating several intermediates, thus saving the losses incident to their isolation, as well as losses due to the instability of the intermediates.

When the original Ainley-King compound was retested it showed definite promise as an antimalarial. Because of this a large number of such compounds have been prepared, both by Dr. Koepfli's and Dr. Buchman's groups here at the California Institute of Technology, and by groups working at Stanford University, Oregon State College, the University of Southern California, and the University of California at Los Angeles[4]. This thesis reports the synthesis of four such compounds.

One modification in the original Ainley-King pattern was introduced as a result of the study by Koepfli and Mead[5] of the structure of an *in vivo* degradation product of quinine isolated from rabbit liver by Kelsey, Geiling, Oldham, and Dearborn[6]. This was shown to be 2'-hydroxy-6'-methoxy-3-vinyl-ruban-9-ol, a carbostyryl derivative obtained from quinine by oxidation at the 2-position of the quinoline nucleus. Dr. Koepfli suggested that blocking of this position by some oxidation-resisting group in a quinine-type drug might interfere with detoxication and slow down the elimination of the drug from the body. Mr. Rapport therefore prepared a compound with a phenyl group in this position, 2-phenyl-α-(2-piperidyl)-4-quinolinemethanol (III). The phenyl group produce a forty-fold increase in activity and was included in most of the compounds prepared subsequently.

In this thesis the preparation of four carbinols is described (IV, V, VI, VII). Their formulas are given in the accompanying table together with their activities against P. lophurae infections in ducks[8]. The values given are the activities, Q, of the compounds relative to an equal weight of quinine, both calculated as free bases. For comparison there are included the activities of several other members of the series (I, III, VIII).

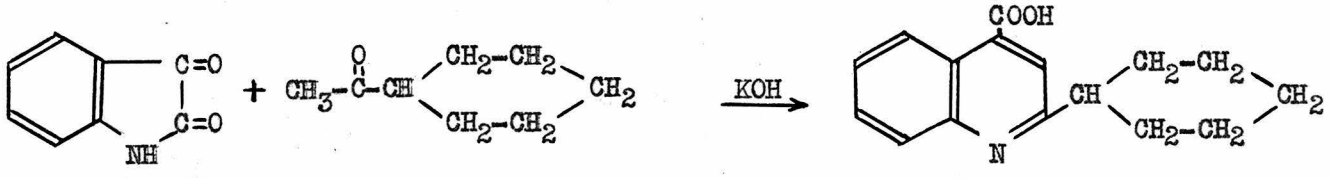
A comparison of the activity of IV with that of V again indicates the significance of the 2-phenyl group. The most active compound prepared in the whole series to date is 6,8-dichloro-2-(p-chlorophenyl)-α-(2-piperidyl)-4-quinolinemethanol prepared by Mr. Meyers[9] under Dr. Buchman's direction. Since it is a general phenomenon in this series that the activity increases when more chlorine molecules are introduced, the 6,7,8-trichloro-2-phenyl compound was prepared to find out if shifting a chlorine in VIII from the para position of the phenyl group to the 7-position in the ring would affect the activity. The 2-cyclohexyl compound, VI, was prepared as a part of an investigation into the effects of replacing the 2-phenyl group with aliphatic rests.

In the four compounds whose preparation is described here the Sargent modification of the Ainley-King method was employed, although each case demanded slightly different conditions. The ethyl ω-benzamidocaproate used was furnished in part by Dr. Elderfield of Columbia University and in part by Dr. Price of the University of Illinois. Three of the cinchoninic esters required were prepared by the Pfitzinger method[10] and the fourth by the Doebner synthesis[11].

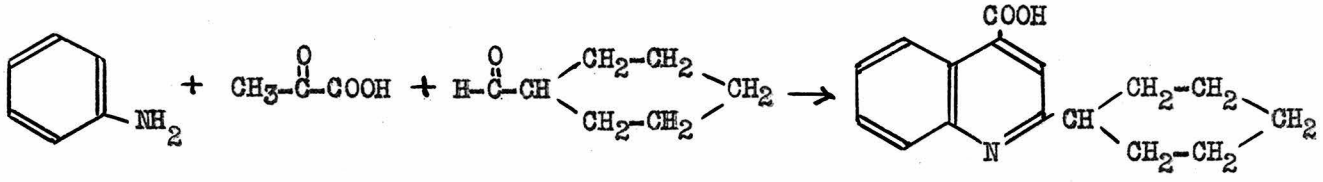
EXPERIMENTAL

The Preparation of Ethyl 2-Cyclohexylcinchoninate

The preparation of 2-cyclohexylcinchoninic acid has been described twice previously, but the melting points of the products obtained by the investigators were very different. John and Pietsch[12] obtained an acid melting at 189° by means of the Pfitzinger reaction between isatin and methyl cyclohexyl ketone.

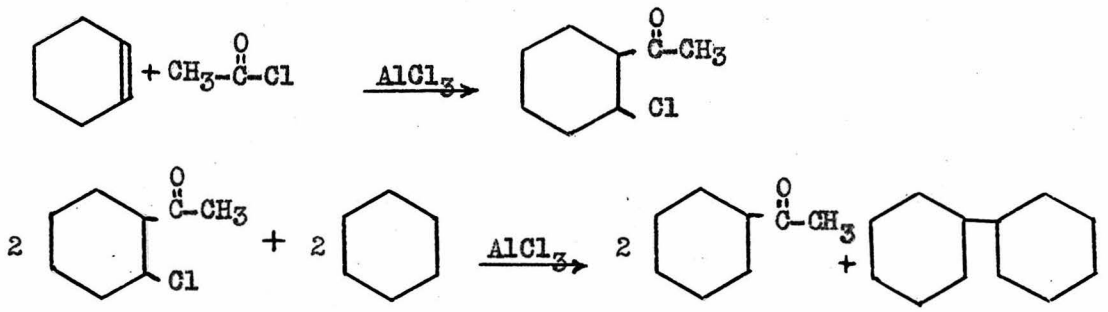


Skita and Wulff[13] prepared this acid by the Doebner reaction of aniline, pyruvic acid, and hexahydrobenzaldehyde.



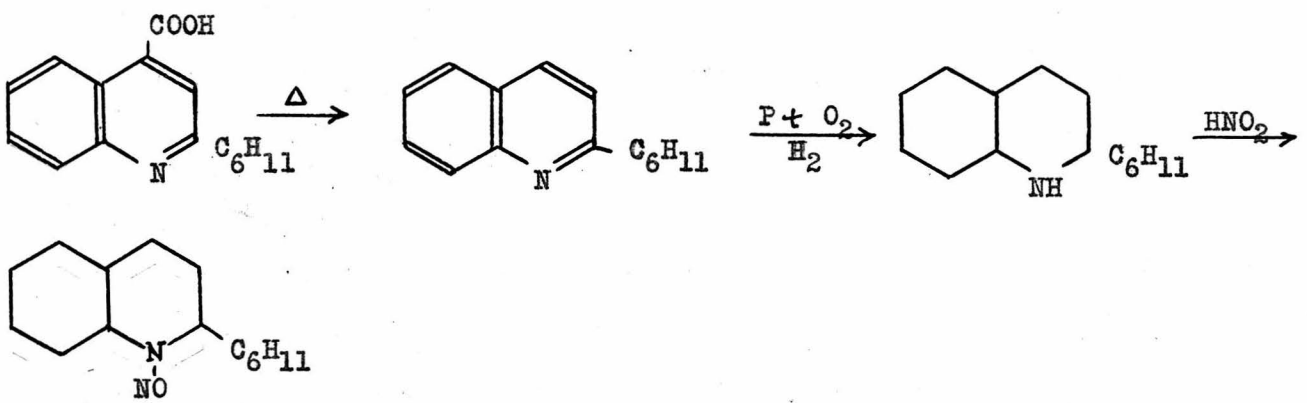
Their product was described as melting at 137°. Because of this confusion an investigation of the product of John and Pietsch was carried out.

Methyl cyclohexyl ketone was conveniently prepared by the method of Nenitzescu and Cioranescu[14].



This was then reacted with isatin as described by John and Pietsch. The melting point of the product showed large variations in different preparations, and depended on the solvent used for crystallization.

The picrates of the methyl and ethyl esters of this acid gave the same melting points described by John and Pietsch. In addition the acid was decarboxylated by Dr. Mead.



The picrate of the 2-cyclohexylquinoline obtained had the melting point reported by John and Pietsch. The free base had a boiling point of 200°/25 mm., while Skita and Wulff gave 180-183°/20 mm. as the boiling point of the corresponding derivative of their acid. Catalytic reduction using platinum oxide as a catalyst caused the base to take up five mols of hydrogen. The nitroso

derivative of the reduction product had a melting point of 185-187°. Skita and Wulff reported 179° as the melting point of a similar nitroso compound from their acid.

A sample of pure acid was prepared by hydrolysing a distilled portion of the ethyl ester. The acid as obtained on precipitation from aqueous solution melted to a gel at 103-108° and became a clear liquid by 168°. After drying in vacuo at 56° for several hours it sintered from 108-160° and melted from 160-172°. A sample recrystallized from toluene melted at 161-177°, one from absolute ethanol melted at 180-184°, while a sample from 50% ethanol melted at 134-141° after sintering for five degrees.

On the basis of this behavior it appears that the acid may be obtained either in an anhydrous form with a melting point as described by John and Pietsch, or in the form of a hydrate melting much lower. The compound described by Skita and Wulff is probably this hydrate, although their analytical results indicate that the water was removed by drying before analysis. It is not excluded that these are two entirely distinct compounds. As the Pfitzinger synthesis is more straightforward than that of Doebner, and as the method used by Skita and Wulff to prepare the hexahydrobenzaldehyde was very devious, in the event of a discrepancy it seems certain that the compound as prepared is the true 2-cyclohexylcinchoninic acid.

Methyl cyclohexyl ketone. - In a three neck flask immersed in an isopropyl ether bath maintained at -20° with dry ice were placed 202 g. of cyclohexene, 192 g. of acetyl chloride, and 500 ml. of cyclohexane. The solution was stirred vigorously while 654 g. of reagent aluminum chloride was added at such a rate that no evolution of hydrogen chloride took place. This required one hour. Towards the end the mixture became so thick that stirring was no longer

possible. After the addition was complete the mixture stood at -20° for one hour, and was then allowed to warm to room temperature. Rapid evolution of hydrogen chloride occurred. The resulting red oil was warmed for several hours on a steam bath, cooled, and poured over ice. A vigorous reaction ensued.

The organic phase was separated, and the aqueous phase was extracted twice with ethyl ether. The combined organic solutions were distilled at atmospheric pressure. The fraction boiling from 100-230° was collected and fractionated at reduced pressure using a 10-cm. column packed with glass rings. A fraction boiling at 73-75°/38 mm. was collected. It weighed 131 g., a yield of 42% of material pure enough for the next reaction.

2-Cyclohexylcinchoninic acid. - A mixture of 129.6 g. of methyl cyclohexyl ketone, 162 g. of isatin, and 220 g. of potassium hydroxide in 400 ml. of water was heated on a water bath for twenty-four hours. At this time 25 g. of isatin was added, and the heating continued for two days more. The solution was diluted to 3 l. with water, and extracted with ethyl ether to remove unreacted ketone. The aqueous solution was warmed and brought to pH 3 with glacial acetic acid. The precipitated acid was filtered, washed with water, and dissolved in 2 l. of hot toluene. The product refused to crystallize from toluene, so it was extracted with 5% sodium hydroxide solution and reprecipitated with hydrochloric acid. On recrystallization from absolute ethanol 140 g. of brown powder, m.p. 173-177° was obtained. This is a yield of 53%. From the mother liquors on the addition of water 36 g. of material melting to a gel at 103-108° was obtained, an additional 13% calculated as a hydrate.

Methyl 2-cyclohexylcinchoninate picrate. - A small amount of 2-cyclohexylcinchoninic acid was esterified with methanol containing a few milliliters of sulfuric acid. The red oily ester was dissolved in ethanol and an

ethanolic solution of picric acid added. The yellow crystalline precipitate melted at 133-134° after recrystallization from ethanol. John and Pietsch report 135° as the melting point of their picrate.

Ethyl 2-cyclohexylcinchoninate. - One hundred forty grams of anhydrous 2-cyclohexylcinchoninic acid was suspended in 1 l. of absolute ethanol and 100 ml. of concentrated sulfuric acid was added. After refluxing the resulting solution for sixteen hours the ethanol was removed at the water pump, ice and water were added, the mixture was made basic with ammonia, and the red oil thus liberated taken up in ethyl ether. After drying the ethereal solution with calcium chloride the ether was stripped off, and the ester distilled. One hundred twenty and three-tenths g., 77% of a light red liquid boiling at 165-168°/225 μ was collected.

Ethyl 2-cyclohexylcinchoninate picrate. - This was prepared in the same way as the methyl ester picrate. It melted at 155.5-157°. John and Pietsch report 157° for the melting point of this compound.

Hydrolysis of ethyl 2-cyclohexylcinchoninate. - Into a solution of 4 ml. of sulfuric acid in 8 ml. of water was introduced 2.06 g. of ethyl 2-cyclohexylcinchoninate. On warming the ester dissolved. After refluxing for one hour the solution was diluted to 50 ml. with water. The resulting suspension was made basic with ammonia, and then brought to pH 3 with glacial acetic acid. The solid precipitate was filtered and dried on a porous plate. It consisted of 1.8 g. of a fluffy white powder which sintered from 98-103°, melted to a glass from 103-108°, and became clear at 168°.

A sample dried two hours over P_2O_5 in a vacuum at 56° sintered from 110-160° and melted from 160-172°. Crystallization from toluene, after boiling to remove any water, gave a melting point of 161-177°, while from absolute ethanol the material melted at 180-184°. From 50% ethanol it sintered from 127-134° and melted at 134-141°.

The Preparation of 2-Cyclohexyl- α-(2-piperidyl)-4-quinoline-methanol Dihydrochloride

This compound was prepared from ethyl 2-cyclohexylcinchoninate and ethyl ω-benzamidocaproate following the usual modified Ainley-King method. The overall yield was 47% on the basis of the ester consumed. Some difficulty was experienced in obtaining reproducible analytical data for the hydrochloride of the final carbinol, but the evidence shows that it was a hydrate, and the analytical figures on the best sample prepared indicate that it existed as a monohydrate.

ε-Bromo-ε-(2-cyclohexylcinchonyl)-n-amylamine dihydrobromide. - In a 500 ml. round bottom flask sodamide was prepared from 11.5 g. of sodium as described for compound IV. The flask was equipped with a reflux condenser and a Hershberg stirrer. To the sodamide was added a solution of 89.5 g. of ethyl 2-cyclohexylcinchoninate and 86.5 g. of ethyl ω-benzamidocaproate in 140 ml. of dry benzene. The viscous mass was warmed in an oil bath to 100°. Following the initial evolution of ammonia the mixture set to a solid in about an hour. After several hours it became possible to start the stirrer again and the brown oil was heated at 100° so that the total time of heating was twenty hours.

The oil was transferred to a 1 l. flask and 450 ml. of 6N hydrochloric acid was added. The two phase mixture resulting was distilled until benzene ceased coming over, the loss in volume being made up by adding hydrochloric acid. Then the mixture was refluxed for sixty-seven hours at which time a homogeneous solution existed.

The solution was cooled in an ice-salt bath and after the addition of 300 ml. of chloroform and 300 ml. of water it was made basic with 50% sodium

hydroxide solution, the temperature being maintained below 25°. The chloroform layer was removed, the aqueous layer re-extracted with chloroform, and the combined chloroform solutions added to 330 g. of 40% hydrobromic acid.

After thorough mixing the hydrobromic acid layer was separated and heated to drive out dissolved chloroform. It had increased in weight by 47 g. The weight of bromine equivalent to 47 g. of a free aminoketone, i.e., 23.5 g., was dissolved in about 35 ml. of 48% hydrobromic acid. The solution of the ketone was then heated to 80° and the bromine added dropwise with shaking during a ten minute period. The solution was heated for ten additional minutes and allowed to cool.

To isolate the bromketone the hydrobromic acid was removed by distillation <u>in vacuo</u> over steam. The residual thick black oil was taken up in 300 ml. of isopropanol and again evaporated to dryness <u>in vacuo</u>. It was then redissolved in 300 ml. of isopropanol, treated with Norite, and placed in the cold room. On standing overnight white crystals appeared.

The product was filtered and washed with isopropanol till the washings were colorless, and then rinsed with acetone. Upon air drying 56.2 g. of material melting at 145-150° was obtained.

An analytical sample recrystallized from isopropanol melted with effervescence at about 145° in a fashion suggesting it was a solvate.

Anal. Calcd. for $C_{21}H_{29}N_2OBr_3$ C_3H_8O (625.3): C, 46.09%; H, 5.96%.

Found: C, 45.80%; H, 5.82%.

By acidification of the aqueous solution after the chloroform extraction, and subsequent separation of 2-cyclohexylcinchoninic acid from precipitated benzoic acid by washing with ether and recrystallization from

ethanol, there were recovered 40.7 g. of 2-cyclohexylcinchoninic acid. On the basis of ester consumed the yield of bromketone salt was 57%, or 28% on the basis of ester originally taken.

2-Cyclohexyl-α-(2-piperidyl)-4-quinolinemethanol (SN 10749). - Fifty-five and two-tenths grams of the bromketone dihydrobromide was suspended in 600 ml. of absolute ethanol in a 2 l. flask. Two hundred and fifty ml. of saturated sodium carbonate solution was added and the mixture shaken for one hour, after the air in the flask had been replaced with nitrogen. Then one gram of Adams platinum oxide catalyst suspended in 100 ml. of absolute ethanol was added. The solution was then shaken under hydrogen until absorption of hydrogen ceased. This required one hour.

The suspended inorganic salts and the catalyst were removed by filtration and the residue washed thoroughly with ethanol. The filtrate and washings were stripped of ethanol *in vacuo*, leaving a tan gum and as aqueous solution. The gum was dissolved in 250 ml. of chloroform and washed with water. The chloroform layer was evaporated to dryness *in vacuo* and the residue dissolved in 75 ml. of warm absolute alcohol. To the solution 70 ml. of saturated ethanolic hydrogen chloride was added. The product crystallized out on standing.

The crystals were filtered and washed with a little absolute ethanol, and with two small portions of acetone. They were dried in air and *in vacuo* over sodium hydroxide. This gave a grey powder melting at 176-181° and weighing 30.0 g., a yield of 82%.

For purification the product was recrystallized from 375 ml. of absolute ethanol. This gave 17.5 g. of microcrystalline solid, m. p. 177-180°. By

concentrating the mother liquors and adding isopropyl ether an additional 7.1 g. were obtained.

The free carbinol was prepared by dissolving a sample of the dihydrochloride in methanol and basifying with aqueous sodium hydroxide. The methanol was evaporated off and the oily residue washed with water. The residue was taken up in ethanol and evaporated down several times to dry it and then taken up in ligroin with a trace of methanol. On standing overnight it crystallized out in rosettes of white prisms. Recrystallized from acetonitrile it gave beautiful long needles, m. p. 157-159°.

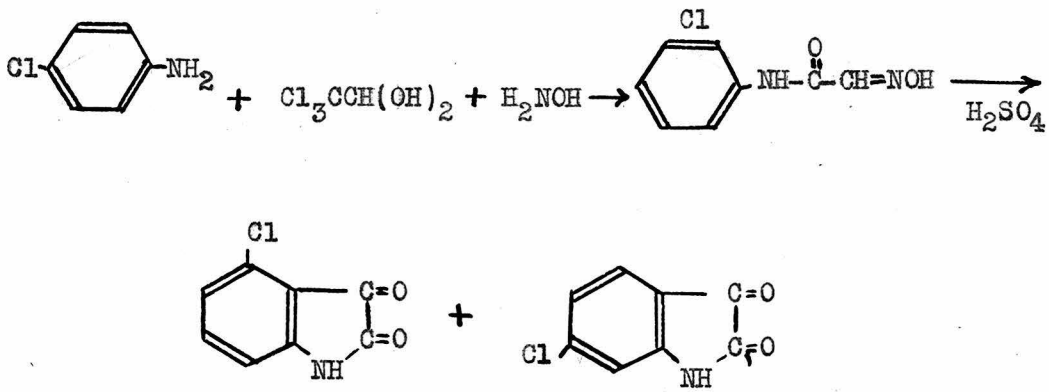
Anal. Calcd. for $C_{21}H_{28}N_2O$ (324.5): C, 77.71%; H, 8.70%; N, 8.64%.
Found: C, 77.53%; H, 8.67%; N, 8.49%.

An analytical sample of the hydrochloride was prepared from the free base by precipitation from ethanolic solution with ethanolic hydrogen chloride. It melted at 178-181° and gave the following results.

Anal. Calcd. for $C_{21}H_{30}N_2OCl_2 \cdot H_2O$ (415.4): C, 60.71%; H, 7.77%.
Found: C, 61.00%; H, 7.87%.

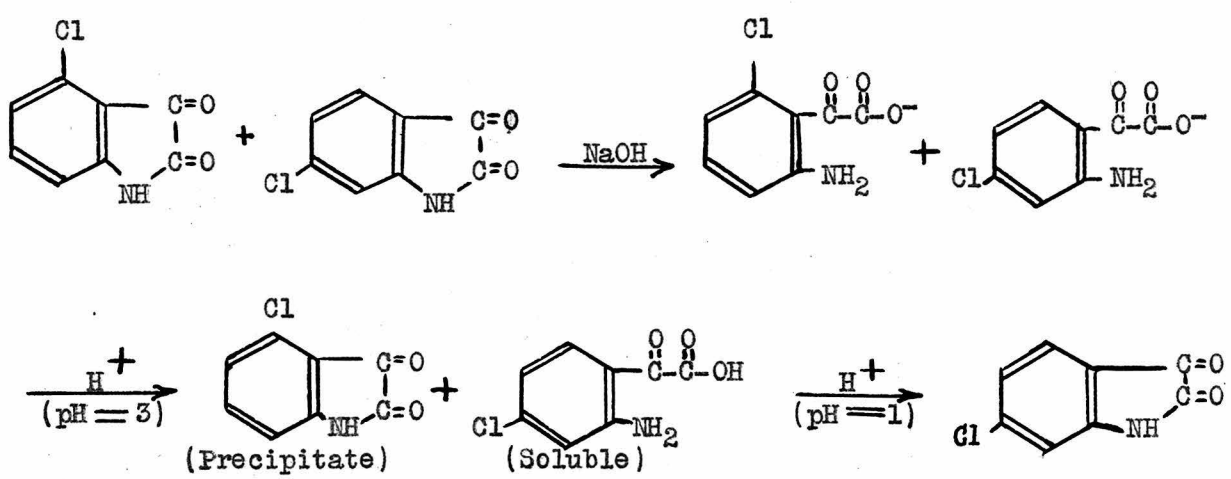
The Preparation and Characterization of 4-Chloroisatin and 6-Chloroisatin

Attention was directed towards the development of suitable laboratory syntheses of these two compounds, since they could be made to undergo the Pfitzinger reaction giving substituted cinchoninic acids which were desired for use in preparing chlorinated Ainley-King compounds. Sandmeyer[15] had previously described the preparation of these compounds by the reaction shown,



but his method led to a mixture of the two isomers which he did not attempt to separate.

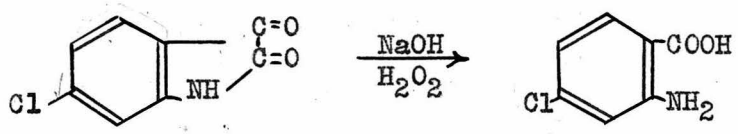
This Sandmeyer synthesis has been carried out on a large scale a number of times and shown to be very satisfactory. The conditions used were similar to those described by Marvel and Hiers[16] for the synthesis of isatin. A method for the separation of the two isomers by fractional precipitation from a basic solution was worked out and it was discovered that they occur



in approximately equal amounts in the mixture.

The decision as to which of the products isolated was the 4-chloroisatin and which the 6-chloro isomer was rendered making use of the method of Sumpter

and Jones[17] of oxidizing isatins to the corresponding anthranilic acids. The



chloro anthranilic acids thus obtained checked as to melting points with those described by Cohn[18] and by Hunn[19].

m-Chloro-isonitrosoacetanilide. - Four hundred and ninety grams of chloral hydrate was dissolved in 8 l. of water in a 22-l. flask, and 3110 g. of anhydrous sodium sulfate was added and caused to dissolve by gentle warming and stirring. A solution of 342 g. of m-chloroaniline in 1600 ml. of water and 235 ml. of concentrated hydrochloric acid was added. A white curdy precipitate formed. Then a solution of 590 g. of hydroxylamine hydrochloride in 1 l. of water was added.

The suspension was heated to boiling during the course of forty-five minutes. The original precipitate slowly dissolved, and at about 70° the product began to come out as a flocculent solid. By the time the boiling point was reached the suspension was so thick that even with vigorous stirring it was very difficult to prevent frothing. The mixture was boiled for several minutes and allowed to cool.

When the mixture had cooled to room temperature 4 l. of ethyl ether was added, dissolving the solid. This ether solution was separated from the aqueous solution, filtered, and the ether was allowed to evaporate in an open pan. There was left 493 g. of a light tan solid. This was a yield of 93%.

A sample purified by reprecipitation from sodium hydroxide solution and

recrystallization from benzene melted at 144-146° (dec). The material as isolated, however, was pure enough for the next step without further work.

4-Chloroisatin and 6-chloroisatin. - The material from the previous reaction was ground in a mortar and then added in portions to 2.5 l. of concentrated C. P. sulfuric acid, which was stirred vigorously and maintained at 80-85° with a cold water bath. The addition required about twenty minutes due to the large heat of reaction. Then the resulting deep red solution was heated for fifteen minutes at 90-95° and poured over a large quantity of cracked ice.

The red solid which precipitated was filtered off, and used directly for the separation of the isomers. This mixture on drying showed a melting range of about 203-210°.

Separation of 4-chloroisatin and 6-chloroisatin. - The moist filter cake was placed in a 12-l. flask together with 5 l. of water. Upon the addition of a solution of 120 g. of sodium hydroxide in 1 l. of water the isatin dissolved giving a deep red color. The solution was filtered through a layer of Celite to remove a small amount of muck. The clear red filtrate was cautiously brought to neutrality with concentrated hydrochloric acid while stirring rapidly. At the point where solid just began to precipitate (about pH 8) a solution of 100 ml. of concentrated hydrochloric acid in 500 ml. of water was added. An orange precipitate of 4-chloroisatin (see below) formed at once and was filtered off after five minutes. To the filtrate was added 500 ml. of concentrated hydrochloric acid causing 6-chloroisatin to come out in the form of small orange plates. This also was removed by filtration.

The first precipitate weighed 224 g., 46%, on the basis of m-chloroaniline,

and melted at 238-248° after sintering for ten degrees. It still contained a small amount of the other isomer, and for careful work required recrystallization before use. Glacial acetic acid was a very suitable solvent. An analytical sample recrystallized from acetic acid consisted of red needles, m. p. 256.5-258°.

Anal. Calcd. for $C_8H_4NO_2Cl$ (181.6): C, 52.89%; H, 2.22%; N, 7.71%.
Found: C, 52.55%; H, 2.32%; N, 7.64%.

The second precipitate weighed 145 g., a yield of 30% based on m-chloroaniline. It melted at 253-257° after sintering for four degrees, and was quite pure although it could be recrystallized from acetic acid if desired. An analytical sample from glacial acetic acid melted at 258-259° and gave the following results.

Anal. Calcd. for $C_8H_4NO_2Cl$ (181.6): C, 52.89%; H, 2.22%; N, 7.71%.
Found: C, 53.04%; H, 2.23%; N, 7.97%.

Structure proof for 6-chloroisatin. - Five grams of a recrystallized sample of the second precipitate above was mixed with 150 ml. of 10% hydroxide solution. The solid partly dissolved to give a red solution which turned green as the isatin ring opened. Then 150 ml. of 3% hydrogen peroxide solution was added. The remaining solid dissolved. After standing one half hour the solution was made acid with hydrochloric acid, precipitating a white solid. This weighed 3.6 g., a yield of 76%. A sample twice recrystallized from ethanol melted at 237-238° and analyzed for a chloroanthranilic acid. Cohn[18] gives 235-236° as the melting point of 4-chloroanthranilic acid, while Hunn[19] gives 240° (corr.).

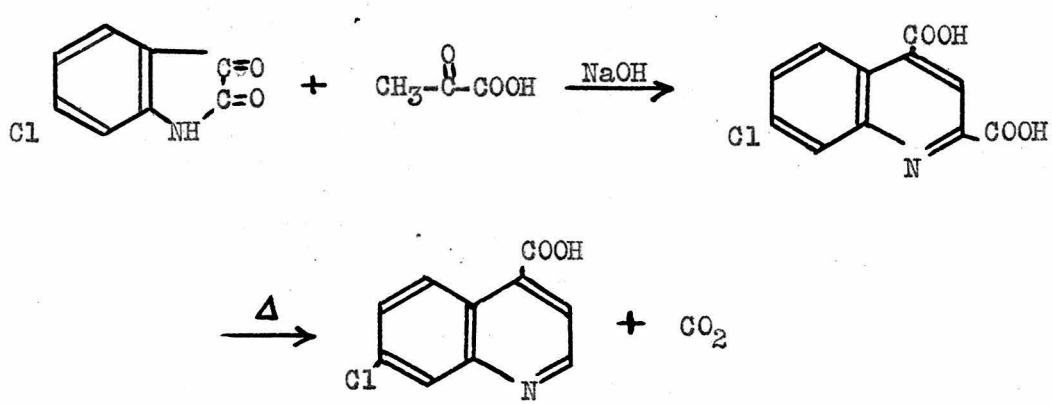
Anal. Calcd. for $C_7H_6NO_2Cl$ (171.6): C, 48.99%; H, 3.53%.
Found: C, 49.44%; H, 3.81%.

As a check the acetyl derivative was prepared by dissolving a sample of the acid in acetic acid, adding acetic anhydride and a trace of sulfuric acid, and isolating the product by pouring the resulting mass into water. The product recrystallized from aqueous ethanol melted at 212-215°. Cohn gives 214° as the melting point of 4-chloroacetanthranilic acid.

Structure proof for 4-chloroisatin. - Five grams of a recrystallized sample of the first precipitate was treated in a similar fashion. On acidification 750 mg. of red solid came out which proved to be unchanged 4-chloroisatin. The solution was treated with Norite and extracted with ethyl ether. On removing the ether 1.7 g. of a brown solid was left, m. p. 141-143°. On recrystallization from aqueous ethanol it came out in long thick brown plates, m. p. 140-141° (dec). Cohn[18] gives 146-147° for the melting point of 6-chloroanthranilic acid.

The Preparation of Ethyl 7-Chlorocinchoninate

The preparation of 7-chloroquinoline-2,4-dicarboxylic acid from 6-chloroisatin and pyruvic acid by the Pfitzinger reaction[10] presented no difficulties. The decarboxylation of this acid to give 7-chlorocinchoninic acid was also



satisfactory provided care was taken to ensure that the dicarboxylic acid was completely freed from sodium ions on precipitation. On neutralizing basic

solutions of quinoline-2,4-dicarboxylic acids there is some tendency for the precipitation of a mono-sodium salt which will not undergo decarboxylation. To avoid this digestion of the precipitated acid with hydrochloric acid was introduced.

7-Chloroquinoline-2,4-dicarboxylic acid. - Sixty-three and four-tenths grams of finely ground 6-chloroisatin was suspended in a solution of 455 g. of sodium hydroxide in 1100 ml. of water. The suspension was warmed to 100° until the original purple color due to the sodium salt of the isatin had disappeared, leaving an olive suspension. After cooling to roon temperature 127 g. of 50% pyruvic acid in water was added. After having been stirred for an hour the mixture was heated in a boiling-water bath for five hours.

The solid cake which had formed was dissolved in 4 l. of water and the solution was made acid to Congo paper with hydrochloric acid. The solid which precipitated was filtered, washed, digested over steam for six hours with 500 ml. of 6N hydrochloric acid, refiltered, washed acid-free, and dried. This yielded 83.1 g., 95%, of a grey solid which melted at 282-284°. An analytical sample crystallized from acetic acid consisted of colorless thin rectangular platelets which melted at 285-290° (dec) after showing a change in structure at 238°.

Anal. Calcd. for $C_{11}H_6NO_4Cl$ (251.6): C, 52.50%; H, 2.41%; N, 5.56%.
Found: C, 52.45%; H, 2.54%; N, 5.65%.

The dimethyl ester was prepared by dissolving the acid in methanol saturated with hydrogen chloride. It crystallized from ethanol in poorly formed plates, m. p. 130-131°.

Anal. Calcd. for $C_{13}H_{10}NO_4Cl$ (279.7): C, 55.81%; H, 3.60%; N, 5.01%.
Found: C, 55.71%; H, 3.61%; N, 5.21%.

The diethyl ester was prepared by Mr. Mislow[20]. It melted at 95-96°.

Anal. Calcd. for $C_{15}H_{14}NO_4Cl$ (307.7): C, 58.55%; H, 4.59%; N, 4.55%.

Found: C, 58.69%; H, 4.77%; N, 4.42%.

7-Chlorocinchoninic acid. - Eighty-three and one-tenth grams of 7-chloroquinoline-2,4-dicarboxylic acid was suspended in 300 ml. of dry nitrobenzene and refluxed for one hour, during which period the solid dissolved. After cooling the crystalline solid which came out was filtered, washed free of nitrobenzene with ethyl ether, and dried. The yield was 57.5 g., 84%, of a coal black solid melting at 285-289° (dec.). An analytical sample was prepared by recrystallization from acetic acid and consisted of colorless long platelets melting at 290-291° (dec.).

Anal. Calcd. for $C_{10}H_6NO_2Cl$ (207.6): C, 57.84%; H, 2.91%; N, 6.75%.

Found: C, 57.76%; H, 3.30%; N, 6.32%.

Ethyl 7-chlorocinchoninate. - In 1 l. of absolute ethanol was suspended 55.4 g. of crude 7-chlorocinchoninic acid, and dry hydrogen chloride was bubbled in to saturation. After refluxing for three hours the solvent was stripped off in vacuo leaving a residue of the ester hydrochloride. To this 200 ml. of ice water was added, and the mixture made basic with ammonia. The liberated ester was then dissolved in 300 ml. of 60-70° petroleum ether. The black solution obtained was treated with Norite and the clear yellow filtrate was concentrated to 100 ml. After standing several days in the ice chest, the ester crystallized from this solution in large prisms, m. p. 33-35°. The yield was 48.2 g., 77%. It was possible to distill this ester if desired, with the b. p. 145°/0.7 mm. An analytical sample after both distillation and crystallization melted at 35-36°.

Anal. Calcd. for $C_{12}H_{10}NO_2Cl$ (235.7): C, 61.16%; H, 4.28%; N, 5.94%.

Found: C, 60.92%; H, 4.37%; N, 5.55%.

The picrate of this ester was prepared by precipitation from an ethereal solution with alcoholic picric acid. On recrystallization from ethanol it gave yellow needles, m. p. 173-174°.

Anal. Calcd. for $C_{18}H_{13}N_4O_9Cl$ (464.8): C, 46.51%; H, 2.82%.

Found: C, 46.22%; H, 2.91%.

A sample of the ester hydrochloride recrystallized from ethanol consisted of colorless long needles, m. p. 165.5-168°.

The Preparation of 7-Chloro- α-(2-piperidyl)-4-quinolinemethanol Dihydrochloride

ε-Bromo- ε-(7-chlorocinchonyl)-n-amylamine Dihydrobromide. - Sodamide was prepared by dissolving 16.5 g. of sodium in liquid ammonia and adding ferric chloride as a catalyst. When the blue sodium color had disappeared the excess ammonia was driven off and the grey sodamide was crushed.

The two liter flask used for this was equipped with a Hershberg stirrer driven by a powerful motor and with a reflux condenser. At room temperature was added a solution of 155 g. of ethyl ω-benzamidocaproate and 138 g. of ethyl 7-chlorocinchoninate* in 325 ml. of benzene. On heating slowly a grey paste first formed which set to a hard mass when the bath temperature approached 80°. The temperature was held constant at 100° and after an hour the mass began to turn oily. After two hours it was possible to start the stirrer.

*The ester used in this run was prepared by Mr. Kurt Mislow.

After seventeen hours of heating the mixture had separated into two phases, a clear benzene phase and a viscous oil. At this time a solution of 435 ml. of concentrated hydrochloric acid and 325 ml. of water was added and the benzene was distilled out. The oily suspension was refluxed for thirty-seven hours, with the temperature of the refluxing vapors at 107°. During this time the red oil slowly went into solution in the acid phase so that at the end of twenty-four hours a clear solution had been obtained.

This was now cooled in an ice-salt bath and cautiously made basic with 50% sodium hydroxide solution, the temperature being held below 20°. The basic solution was now extracted three times with chloroform. The combined chloroform solutions were placed in a flask containing 490 g. of 48% hydrobromic acid and 435 g. of water. The weight of the flask and the hydrobromic acid were known accurately.

When extraction of basic material from the chloroform layer was complete the chloroform was siphoned off and discarded; then the acid solution was heated to drive out dissolved chloroform, a stream of nitrogen passing through the flask being used to help this process.

The increase in weight of the hydrobromic acid solution was 104 g., the equivalent of 0.376 mols of ketone. A solution of 53.2 g., 0.335 mols, of bromine in 50 ml. of 48% hydrobromic acid was prepared and added slowly to the acid solution of the product at a temperature of about 80°. This required ten minutes. A yellow oil formed momentarily and then went into solution. After addition was completed the solution remained turbid due to undissolved oil, but after heating for twenty minutes further most of this had disappeared. The solution was filtered through glass wool to remove traces of oil, and was then allowed to cool. The product crystallized out in a mass of fine needles. It was filtered, washed with acetone and ether, and air dried, giving 86 g.

of yellow, crystalline material which melted with decomposition at 190-193°. On further standing an additional 18 g. of product crystallized out, while a second crop was obtained by concentrating the hydrobromic acid mother liquors and adding an equal volume of acetone. It weighed 37 g. and appeared to be as pure as the first crop. The total yield was thus 141 g. of bromketone, or 47%. By acidification of the basic solution from the condensation and working up of the precipitated acids 37 g. of 7-chlorocinchoninic acid was recovered accounting for an additional 30% of the starting material.

An analytical sample of this compound was prepared by crystallization from dilute hydrobromic acid. It decomposed at 184-186°.

Anal. Calcd. for $C_{15}H_{18}N_2OClBr_3$ (517.5): C, 34.83%; H, 3.51%; N, 5.41%.
Found: C, 35.17%; H, 3.67%; N, 5.07%.

7-Chloro-α-(2-piperidyl)-4-quinolinemethanol dihydrochloride (SN 8153). - Eighty-six grams of the first crop of bromketone dihydrobromide was placed in a flask with 840 ml. of redistilled methanol and 385 ml. of saturated sodium carbonate solution (d 1.15) was added. Air was swept out of the flask with a stream of nitrogen and the flask was stoppered and shaken for one hour. Then 3.5 g. of Adams platinum oxide catalyst was added and the mixture was shaken under hydrogen until the absorption of gas ceased. This required about six hours.

The catalyst and inorganic salts were filtered from the solution and washed thoroughly with methanol. The filtrate and washings were concentrated in vacuo to remove the methanol, leaving a red oil and an aqueous phase. The oil was taken up in chloroform and separated from the aqueous phase, the chloroform solution filtered, and the solvent removed. The residual red oil was dissolved in 150 ml. of absolute ethanol and dry hydrogen chloride was passed into the solution until it was saturated. A pink crystalline precipitate formed which was filtered, washed with ethanol and ether, air dried. It was a reddish

powder weighing 19.9 g., 34 %, which melted with decomposition at 191-193° after darkening from 180°.

For purification the crude carbinol dihydrochloride was recrystallized from 560 ml. of methanol with the aid of Norite. On cooling a first crop of 9.0 g. of colorless, wedge-like crystals came out melting at 206-209° (dec). On concentrating the mother liquors a second crop of 6.4 g. of equally pure material was obtained. This is a 26% yield of recrystallized material.

The 55 g. of second crop bromketone salt from the above preparation was reacted in the same way. It gave 10.2 g. of crude dihydrochloride and 7.8 g. of recrystallized product.

The overall yield on this experiment of 23.2 g. of recrystallized product is 11.3% on the basis of the cinchoninic ester used, or 16.3% on the basis of the ester consumed.

An analytical somaple of the carbinol dihydrochloride was recrystallized several times from methanol. It melted with decomposition at 207-208° after darkening for fifteen degrees proviously.

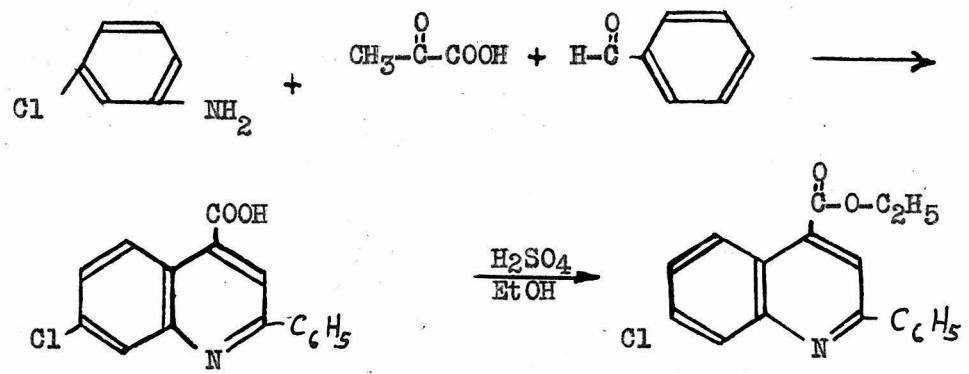
Anal. Calcd. for $C_{15}H_{19}N_2OCl_3$ (349.7): C, 51.54%; H, 5.48%; N, 8.01%.
Found: C, 51.08%; H, 5.38%; N, 7.79%.

The free base was prepared for analysis by suspending the carbinol dihydrochloride in water and adding excess sodium hydroxide solution. The precipitated base was centrifuged down, washed with water, and recrystallized several times from 95% ethanol. It showed a change in structure around 130° and melted at 173-174°.

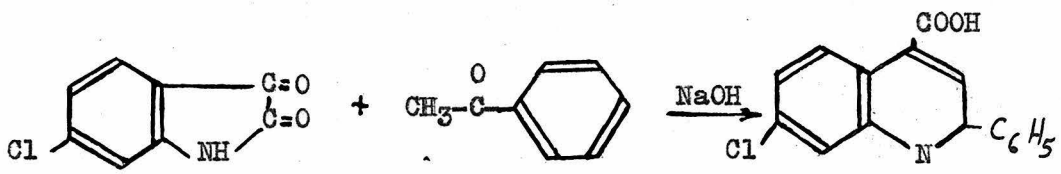
Anal. Calcd. for $C_{15}H_{17}N_2OCl$ (276.7): C, 65.13%; H, 6.19%; N, 10.13%.
Found: C, 65.17%; H, 6.47%; N, 10.18%.

The Preparation of Ethyl 7-Chloro-2-phenylcinchoninate

The preparation of 7-chloro-2-phenylcinchoninic acid has been described by Borsche[21], using the Doebner reaction of m-chloroaniline, benzaldehyde, and pyruvic acid. This was carried out on a large scale and proved to be very



satisfactory. Since the possibility exists that this reaction might lead to 5-chloro-2-phenylcinchoninic acid instead of the 7-isomer the acid was also synthesized by the Pfitzinger reaction from 6-chloroisatin and acetophenone, eliminating any doubt as to the position of the chlorine. The acids obtained



in these two syntheses were identical, a conclusion which was confirmed by a comparison of the esters prepared from them.

The esterification of the acid was simple and straightforward. Since the completion of this work Tarbell and his co-workers[22] have described the

preparation of this ester. The melting point they gave is the same as that reported here.

7-Chloro-2-phenylcinchoninic acid. - Three liters of U. S. P. glacial acetic acid was placed in a five-liter flask equipped with a stirrer. While stirring 220 g. of redistilled benzaldehyde, 360 g. of 50% aqueous pyruvic acid, and 250 g. of m-chloroaniline were added. The resulting red solution was heated on a water bath for one and one-half hours. After thirty minutes yellow needles began to form, and on cooling the amount of these increased. They were filtered, washed with acetic acid and ether, and dried. There was obtained 175 g., 35%, of rectangular prisms, m. p. 249-254° after sintering from 190°. Borsche[21] gives 244-246° for this melting point.

Ethyl 7-chloro-2-phenylcinchoninate. - The 175 g. of acid were placed in a flask with 1.5 l. of absolute ethanol and 100 ml. of sulfuric acid. On heating the solid dissolved. The solution was refluxed for nineteen hours and the ethanol was then distilled off in vacuo. On adding ice some solid came out, and when the mass was made basic with ammonia a solid cake formed. This was dissolved in ethyl ether and the solution was extracted thoroughly with ether. The solid all dissolved except for a small amount of debris which was removed by filtration.

After separating the phases the ether was removed leaving a yellow cake. This was dissolved in one liter of boiling 96% ethanol. On cooling a heavy crop of yellow needles formed which were filtered off and air dried. The yield was 154 g. of material melting at 89-91°, 73% of theory. Tarbell[22] gives 89-89.5° as the melting point of this ester. An analytical sample after several recrystallizations from ethanol melted at 89-90° and gave the

following results.

Anal. Calcd. for $C_{18}H_{14}NO_2Cl$ (311.8): C, 69.35%; H, 4.53%; N, 4.49%.

Found: C, 69.59%; H, 4.60%; N, 4.21%.

7-Chloro-2-phenylcinchoninic acid by the Pfitzinger method. - From 9.3 g. of 6-chloroisatin was prepared 6.6 g., 45%, of an acid, m. p. 246-248° after sintering from 238°, by using the Pfitzinger reaction in the manner described for the preparation of 5-chloro-2-phenylcinchoninic acid. A mixture of this product and the acid from Borsche's synthesis showed no melting point depression. The ester prepared from this acid melted at 88-89° and gave no melting point depression when mixed with the ester prepared from Borsche's acid.

The Preparation of 7-Chloro-2-phenyl- α-(2-piperidyl)-4-quinolinemethanol Dihydrochloride

ε-(7-Chloro-2-phenylcinchonyl)- n-amylamine dihydrobromide. - Sodamide was prepared from 12.5 g. of sodium in a three neck two-liter flask as described previously. The sodamide was ground up and the flask was fitted with a Hershberg stirrer and a reflux condenser. To the flask was added a solution of 134 g. of ethyl 7-chloro-2-phenylcinchoninate* and 118 g. of ethyl ω-benzamidocaproate in 250 ml. of dry benzene. The mixture was stirred and warmed in an oil bath. At about 60° the evolution of ammonia commenced and the mass became a grey paste. The temperature was held constant at 100° for eighteen hours during which period the mixture went to a brown oil. Two hundred and forty-five milleliters of concentrated sulfuric acid

*The ester used in this large scale reaction was furnished by Dr. Elderfield of Columbia University.

and 330 ml. of water were added cautiously, the benzene was distilled out, and the water which came over with the benzene was replaced. The resulting murky solution was refluxed for forty-eight hours to remove the benzoyl group.

The solution was cooled and cautiously made basic with 33% potassium hydroxide solution, the temperature being held below 25°. To prevent the precipitation of potassium sulfate an additional 500 ml. of water was added. At a pH of 4 the mixture became oily. The addition of base was continued until the solution was alkaline and then 100 ml. excess was added. Five hundred milleliters of chloroform was added and the aqueous solution was decanted from the lower layer of oil, chloroform, and insoluble salts. The aqueous solution was thoroughly extracted with chloroform and the chloroform added to the lower layer above. This mixture was now extracted with water until all the material present was in solution in water or chloroform.

The chloroform solution was filtered and added to 600 ml. of 20% hydrobromic acid. A yellow solid crystallized out of the two phase mixture, and after standing for two hours was filtered and washed with acetone. After drying in air and <u>in vacuo</u> this was 99.4 g., 45%, of yellow crystals which melted at 264-269° and analyzed for the desired compound, after several recrystallizations from methanol.

Anal. Calcd. for $C_{21}H_{23}N_2OClBr_2$ (514.7): C, 48.97%; H, 4.50%; N, 5.45%.

Found: C, 49.02%; H, 4.77%; N, 5.45%.

The aqueous solution from the chloroform extraction was acidified, precipitating a mixture of acids. These were filtered off, washed, dried, and thoroughly extracted with ethyl ether leaving as a residue 64 g. of 7-

chloro-2-phenylcinchoninic acid, m. p. 246-248°. This represented 52% of the ester taken, making the yield of ketone dihydrobromide 93% on the basis of ester consumed.

ε-Bromo-ε-(7-chloro-2-phenylcinchonyl)-n-amylamine dihydrobromide. - Ninety-six and six-tenths g. of the above ketone dihydrobromide was suspended in 1 l. of 48% hydrobromic acid. The suspension was immersed in a boiling water bath and a solution of 30 g. of bromine in 100 ml. of 48% hydrobromic acid was added over a ten minute period, while stirring rapidly. The suspension was heated for twenty minutes more and cooled.

The yellow solid which came out was filtered, washed with acetone, and dried in air and *in vacuo* over sodium hydroxide. This gave 101.0 g., 91%, of crystalline material melting with decomposition at 264-267°. It analyzed correctly for the desired product.

Anal. Calcd. for $C_{21}H_{22}N_2OClBr_3$ (593.6): C, 42.48%; H, 3.73%; N, 4.72%.
Found: C, 43.00%; H, 3.84%; N, 4.26%.

7-Chloro-2-phenyl-α-(2-piperidyl)-4-quinolinemethanol (SN 10,286). - Ninety-seven and two-tenths grams of this bromketone salt was placed in a 4 l. bottle and 1 l. of ethanol and 420 ml. of saturated sodium carbonate solution (d 1.15) were added. A heavy precipitate of sodium bromide formed at once and caked on the walls of the flask. This was broken up, the air was swept from the bottle with a stream of nitrogen, and the suspension was shaken for one hour. Then 2.3 g. of Adams platinum oxide catalyst was added and the mixture shaken with hydrogen until absorption ceased. After standing overnight the mixture of catalyst and inorganic salts was filtered from the solution and thoroughly washed with ethanol.

The washings and filtrate were concentrated *in vacuo* till all the ethanol had been removed, leaving a clear aqueous solution and a red oil. The oil was taken up in chloroform, filtered, and the chloroform removed. The oil was now taken up in 100 ml. of absolute ethanol and 200 ml. of 5.9 N alcoholic hydrogen chloride solution was added. A pink crystalline solid precipitated. After twenty minutes this was filtered and washed with ethanol and acetone. After drying this was 27,7 g., 40%, of a light pink powder which melted with decomposition at 221-222°.

For purification it was taken up in 500 ml. of boiling methanol and precipitated by the addition of 900 ml. of isopropyl ether. This threw out 24.7 g. of a very slightly colored crystalline solid melting with decomposition at 225-226°. This is a yield of purified material of 36%, and an overall yield of 15% from the starting ester or 31% from the ester consumed.

Anal. Calcd. for $C_{21}H_{23}N_2OCl_3$ (425.8): C, 59.23%; H, 5.44%; N, 6.58%.
Found: C, 59.28%; H, 5.70%; N, 6.35%.

A sample of this salt from a preliminary run was converted to the free base by treating an alcoholic suspension with sodium hydroxide solution. The resulting solid was recrystallized from ethanol several times. It behaved like a solvate on melting; liquefying with gas evolution around 100°, resolidifying, and finally melting at 151-152°. It analyzed for an ethanolate of the free base.

Anal. Calcd. for $C_{21}H_{21}N_2OCl\ C_2H_5OH$ (398.9): C, 69.23%; H, 6.82%; N, 7.02%.
Found: C, 69.30%; H, 6.48%; N, 6.80%.

A sample of the salt from this experiment treated in the same fashion gave a white crystalline solid which melted at 190-191° and

analyzed for the solvent-free base.

Anal. Calcd. for $C_{21}H_{21}N_2OCl$ (352.9): C, 71.45%; H, 6.00%; N, 7.94%.

Found: C, 71.61%; H, 6.12%; N, 7.57%.

Since it was felt that the production of two types of free bases in different runs might indicate the formation of two stereoisomeric mixtures in the two experiments these compounds were investigated. It was found that in one case a sample of the ethanolate had spontaneously changed to the higher melting form during a period of several weeks, while another sample of the solvate after drying in vacuo at 78° for four hours also melted at the higher temperature and gave no depression of the melting point when mixed with the product of the reaction described above. It was not, however, possible to convert the non-solvated form to the solvated form by crystallization from ethanol.

The Attempted Preparation of 5-Chloro-α-(2-piperidyl)-4-quinolinemethanol and 5-Chloro-2-phenyl-α-(2-piperidyl)-4-quinolinemethanol

The attempted preparation of these compounds by the Ainley-King procedure failed, since in each case the reaction between the appropiate cinchoninic ester and ethyl ω-benzamidocaproate could not be made to take place. This is probably a steric effect due to the presence of a chlorine atom in the 5 position next to the ester group. In this connection it is interesting to note that the two cinchoninic acids in question could not be esterified in the usual fashion with ethanol and acid. A similar interference with this reaction by a substituent in the 5 position has been noted by Buchman and his co-workers[9] in the case of ethyl 2-phenyl-5,6-benzocinchoninate which would not condense with ethyl

ω-benzamidocaproate although ethyl 2-phenyl-7,8-benzocinchoninate reacted readily.

The low yield in the Pfitzinger reaction between 4-chloroisatin and pyruvic acid is probably due to the difficulty of isolating the product from the mass of pyruvic acid polymers and not to any deficiency of the reaction itself. After the decarboxylation an attempt to esterify the 5-chlorocinchoninic acid obtained by refluxing overnight with ethanolic sulfuric acid resulted in a quantitative recovery of unchanged acid. To overcome this the use of thionyl chloride with intermediate formation of the acid chloride was introduced.

The preparation of 5-chloro-2-phenylcinchoninic acid by the Pfitzinger reaction went nicely. It was necessary to recrystallize the product before esterification since isolation of the ester obtained from a crude sample of acid proved to be very difficult. Again it was impossible to esterify the acid in the usual fashion and thionyl chloride had to be employed.

When these esters were heated with sodamide and ethyl ω-benzamidocaproate in the usual fashion no change could be noted in the time usually sufficient for complete conversion of the initial grey sodium salt of the caproic ester to a brown oily product. When the heating was continued for several days, or when higher temperatures were employed, the mixture gradually changed to a pitch black oil. Attempts to isolate a β-keto ester from this mess failed, nor could any ketone hydrobromide be isolated after hydrolysis. When the reaction was carried on to the bromination stage the solution which should have contained the ketone reacted with only a negligable amount of bromine.

In view of these results, and of the evidence discussed above, the attempt to prepare these carbinols was dropped.

5-Chloroquinoline-2,4-dicarboxylic acid. - Two hundred and seven grams of 4-chloroisatin was suspended in 1225 ml. of 96% ethanol and 410 g. of 50% pyruvic acid was added. While shaking a solution of 360 g. of potassium hydroxide in 390 ml. of water was added. This gave a deep red solution which was heated under reflux for twenty-eight hours.

On cooling the solution 174 g. of brown crystals came out. These were dissolved in 400 ml. of water, the solution was extracted with ether, and made acid to Congo paper with hydrochloric acid. Eighty-two and five-tenths g. of yellow flocculent solid precipitated. I t was infusible and left an ash on ignition. This was assumed to be the mono-potassium salt of the desired dicarboxylic acid. To convert it to the free acid it was dissolved in 2200 ml. of boiling water and 90 ml. of concentrated hydrochloric acid was added. The free acid crystallized out almost at once and was filtered, washed with water, and dried. It weighed 70.8 g. and melted with gas evolution at 216-218°. If the 82.5 g. represented the mono-potassium salt this is a recovery of 99%. It was assumed that the 175 g. of material originally obtained consisted of a mixture of the di-potassium salt of the acid and potassium pyruvate.

The reaction solution after the crystalline product had been removed was also acidified, precipitating a large mass of red muck. This was worked over by extraction with sodium bicarbonate and precipitation with hydrochloric acid. An additional 15.0 g. of acid were obtained, making a total of 86 g., 30% of theory. An analytical sample was recrystallized from acetic acid and from ethanol. It melted with

evolution of gas at 218-219° and gave the following results.

Anal. Calcd. for $C_{11}H_6NO_4Cl$ (251.6): C, 52.50%; H, 2.41%; N, 5.56%.

Found: C, 52.47%; H, 2.53%; N, 5.28%.

5-Chlorocinchoninic acid. - Fifty-eight grams of 5-chloroquinoline-2,4-dicarboxylic acid was heated in a flask containing 120 ml. of nitrobenzene. At 180° the evolution of carbon dioxide began, and at 210° the reaction proceeded very rapidly and the suspended solid dissolved. After twenty minutes at this temperature the solution was cooled whereupon the product crystallized out. It was filtered off, washed free of nitrobenzene with ether, and air dried. This gave 36.2 g., 76%, of a grey crystalline solid, m. p. 253-254°. An analytical sample after several recrystallizations from ethanol consisted of colorless plates, m. p. 254-255°.

Anal. Calcd. for $C_{10}H_6NO_2Cl$ (207.6): C, 57.84%; H, 2.91%; N, 6.75%.

Found: C, 57.82%; H, 3.06%; N, 6.51%.

Ethyl 5-chlorocinchoninate. - Thirty-four and three-tenths grams of 5-chlorocinchoninic acid was refluxed for two and one-half hours with 220 ml. of purified thionyl chloride. The thionyl chloride was then stripped off in vacuo on a steam bath and 250 ml. of absolute ethanol was added to the crystalline residue. On warming the solid dissolved and the solution was refluxed for four hours.

The ethanol was removed in vacuo, ice and water were added, the solution was made basic with ammonia, and the ester was taken up in ethyl ether. The ether solution was treated with Norite, and the ether removed. The residue was a red oil which crystallized on cooling. It weighed 35.6 g., 91%. For purification the ester was distilled, coming over at 136-139°/170-200μ. The distilled product weighed 29.2 g., 75%, and melted at 64-65°. An analytical sample recrystallized from ligroin melted at 65-65.5°.

Anal. Calcd. for $C_{12}H_{10}NO_2Cl$ (235.7): C, 61.16%; H, 4.28%; N, 5.94%.
Found: C, 60.91%; H, 4.49%; N, 5.80%.

5-Chloro-2-phenylcinchoninic acid. - One hundred and eighty-three grams of crude 4-chloroisatin was placed in a 5 l. flask, and 1225 ml. of 95% ethanol and 220 g. of acetophenone were added. Then a hot solution of 220 g. of potassium hydroxide in 300 ml. of water was added. The suspended isatin dissolved to give a deep purple solution. This was refluxed for forty hours and then the ethanol was driven off on a steam bath. Enough water was added to dissolve the residual solid, and excess acetophenone was extracted with ether. Upon acidifaction of the aqueous layer with hydrochloric acid a brown oil came out which solidified on cooling. This solid was crushed and suspended in 2 l. of water. Upon addition of 100 g. of sodium bicarbonate it dissolved, and a small amount of muck was removed by filtration. Acidification now precipitated 206 g., 72%, of a creamy solid. This was recrystallized from 3 l. of methanol, a second crop being precipitated by addition of water to the mother liquors. The total yield of recrystallized material was 163 g., 57%, of crystals melting at 229-232° with the evolution of gas. An analytical sample consisted of colorless rectangular prisms, m. p. 234-235° (dec).

Anal. Calcd. for $C_{10}H_{10}NO_2Cl$ (283.7): C, 67.78%; H, 3.55%; N, 4.94%.
Found: C, 67.93%; H, 3.57%; N, 4.73%.

Ethyl 2-phenyl-5-chlorocinchoninate. - One hundred and sixty-three grams of recrystallized acid was refluxed for two and one-half hours with 700 ml. of purified thionyl chloride. The acid dissolved almost immediately. At the end of this period the excess thionyl chloride was

removed in vacuo leaving a thick red oily residue. To this was added 750 ml. of absolute ethanol causing a white solid to crystallize out, probably the acid chloride hydrochloride. On refluxing for three hours this dissolved. The ethanol was removed leaving a red oil. Five hundred milleliters of ethyl ether was added, then some ice water, and the water was made basic with ammonia setting the ester free so that it went into the ether phase. The ether phase was washed with water, dried over sodium sulfate, and the ether was distilled off. The dark colored oily residue crystallized readily. It was impossible to recover any unreacted acid from the aqueous phase by acidification.

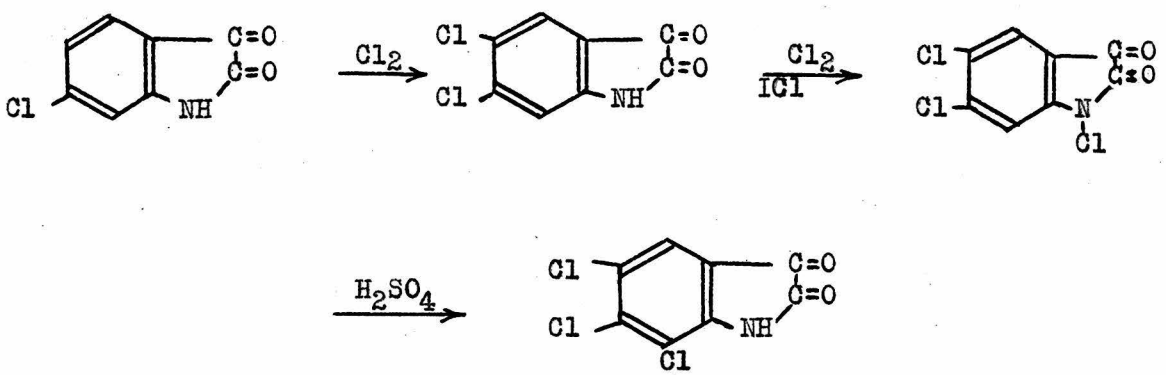
For purification the product was distilled in a high vacuum. This gave 122 g., 68%, of a white crystalline solid melting at 57-59°. It boiled at 194°/140μ and 174°/75μ . An analytical sample crystallized from methanol came out in large white prisms, m. p. 59.5-60.5°.

Anal. Calcd. for $C_{18}H_{14}NO_2Cl$ (311.8): C, 69.35%; H, 4.53%; N, 4.49%.
Found: C, 69.49%; H, 4.23%; N, 4.19%.

The Preparation of Ethyl 6,7,8-Trichloro-2-phenylcinchoninate

In the search for a satisfactory synthesis of this ester two reactions were employed which had previously been used by workers in this laboratory. Rapport[7] had worked out the preparation of 5,6-dichloroisatin and shown it was readily formed by the chlorination of 6-chloroisatin in glacial acetic acid. The reaction of 5-chloroisatin with chlorine in the presence of iodine monochloride to give 1,5-dichloroisatin and the rearrangement of this latter compound to give 5,7-di-

chloroisatin under the influence of sulfuric acid had been described in a German patent[23] and had been explored extensively by Buchman and his co-workers[9] during the preparation of 6,8-dichloro-2-phenylcinchoninic acid. By a combination of these two methods it proved to be possible to prepare the required 5,6,7-trichloroisatin.



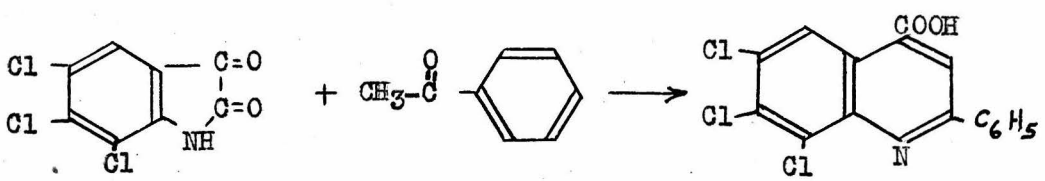
At first the reactions were run successively, i. e., 6-chloroisatin was chlorinated by the Rapport method, 5,6-dichloroisatin was isolated, and then chlorinated further using the German method. It seemed that both reactions might be carried out in one operation and this proved to be so.

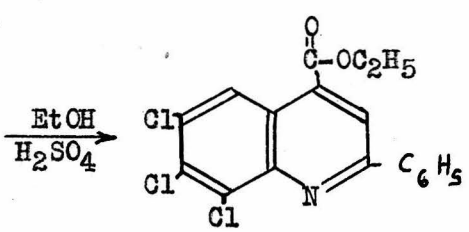
The intermediate N-chloroisatin proved to be impossible to isolate. The solid from the chlorination had a much lower melting point than any of the three chloroisatins involved, but attempts to isolate a pure N-chloro compound apparently led to rearrangement. Hence the crude material was used for the next step.

It proved to be very difficult to free the final 5,6,7-trichloroisatin from various by-products which were formed during the chlorination. While a sufficient amount of pure material was obtained by fractional

crystallization to permit characterization of the compound, during large scale reactions the crude isatin was submitted to the Pfitzinger reaction. One of the principle by-products was a golden compound, nitrogen free and very rich in chlorine, whose structure is unknown.

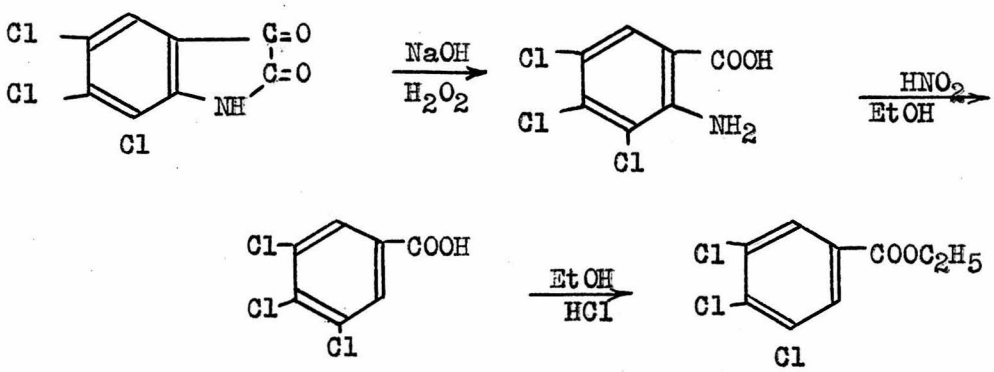
The trichloroisatin was converted to the 2-phenylcinchoninic acid by the Pfitzinger reaction with acetophenone. Again the product obtained proved to be difficult to purify since it was insoluble even in hot, dilute sodium bicarbonate and could only be recrystallized from cellosolve which gave little purification. Therefore, the crude acid was employed for the next step.





The esterification proceeded readily. It was difficult to work up the final product and the yield was not too large. However, it will be noted that the impurities accumulated in all the previous steps added to the difficulty of isolation and to the low yield. Mr. Mislow[20] has recently observed that in place of the isolation procedure described here the ester may be crystallized out by cooling the esterification mixture. The yield is not improved and the ester must be recrystallized, but the ease of handling is much greater.

To prove the structure of the trichloroisatin it was converted to known 3,4,5-trichloroanthranilic acid by the method of Sumpter and Jones[17]. This was not conclusive, however, as 4,5,6-trichloroanthranilic acid which would be formed from the other possible trichloroisatin, the 4,5,6-isomer, has not been described. Therefore the anthranilic acid was deaminated to give 3,4,5-trichlorobenzoic acid which was identified by its melting point and by the melting point of its ester, thus furnishing conclusive proof of the structure of the trichloroisatin.



Since the material employed for the Pfitzinger reaction was an impure mixture one small run was carried out using a purified sample of 5,6,7-trichloroisatin. This demonstrated that the component of the mixture which gave 3,4,5-trichlorobenzoic acid was the same as that which gave the compounds described below.

5,6,7-Trichloroisatin. - One hundred and fifteen grams of crude 6-chloroisatin was suspended in 2.5 l. of water and 25 g. of potassium iodide was added. A rapid stream of chlorine was led into the stirred suspension until the reduction of the iodide ion to iodine monochloride was complete as evidenced by the disappearance of the initial dark purple color. When the water was saturated with chlorine the rate of gas flow was cut down

and the suspension was stirred for seventy-two hours. The solid was then filtered, washed with water, and air dried. This gave 140 g. of brick red solid, m. p. about 140-210°.

The dried solid was added to 600 ml. of C. P. sulfuric acid and held below 50° by cooling with running water. After standing overnight the solution was poured onto cracked ice giving 140 g. of red solid, m. p. 180-195°.

6,7,8-Trichloro-2-phenylcinchoninic acid. - The 140 g. of crude trichloroisatin was added to 1100 ml. of 95% ethanol, 67 g. of acetophenone, and 315 ml. of 33% potassium hydroxide and refluxed for sixteen hours. Two liters of water was added, and then 350 ml. of hydrochloric acid making the solution acid to Congo paper. After cooling a solid was filtered off and dried. It was brick red, weighed 143 g., melted at 269-285° (dec.), and was identical when purified with the analytical sample described below.

Ethyl 6,7,8-trichloro-2-phenylcinchoninate. - The crude acid was refluxed overnight with 1500 ml. of absolute ethanol and 100 ml. of sulfuric acid. Then the ethanol was removed in vacuo, ice was added to the oily residue, and the mixture was made alkaline with concentrated ammonia. The ester was taken up in chloroform and the chloroform was washed with dilute ammonia. The solvent was driven off and the oily residue was digested with 500 ml. of ethanol, thus causing it to crystallize. After cooling the crude ester was filtered off and dried. The ethanol filtrate contained large amounts of red tarry materials from which no ester could be isolated. The solid ester weighed 85 g. and melted at 150-200°.

The solid was distilled in an all glass apparatus packed with glass wool. The ester came over at about 270°/100μ. A great deal of decomposition occurred during the distillation. This may have represented free acid

being decarboxylated to give trichloroquinoline. The distillate was a red oil which crystallized at once. The solid was fused and poured into one liter of boiling ethyl acetate. On cooling the ester crystallized out in stout needles or bars colored light pink or yellow. The yield of purified product was 50 g., 21% from 6-chloroisatin, of material melting at 165-167°. An analytical sample from ethyl acetate melted at 162-165°.

Anal. Calcd. for $C_{18}H_{12}NO_2Cl_3$ (380.7): C, 56.80%; H, 3.18%; N, 3.68%.
Found: C, 56.60%; H, 3.24%; N, 3.94%.

Characterization of 5,6,7-trichloroisatin. - The crude isatin from one run was crystallized from glacial acetic acid, giving a mixture of golden plates and red prisms. Some of the plates were separated by hand and recrystallized for analysis. Found: C, 28.78% (29.10%); H, 0.97% (0.47%); N, 0.00%; Cl, 54.23%.

Some of the red prisms were obtained pure by swirling the flask containing the crystals to suspend the lighter plates and decanting these off and dissolving the residue in hot acetic acid. On cooling some of the prisms came out before the remaining plates crystallized. An analytical sample melted at 248-253°.

Anal. Calcd. for $C_8H_2NO_2Cl_3$ (250.5): C, 38.35%; H, 0.81%.
Found: C, 38.29%; H, 1.09%.

Two grams of this purified trichloroisatin was converted to the cinchoninic acid and ester. From the acid an analytical sample melting at 293-294° was prepared.

Anal. Calcd. for $C_{16}H_8NO_2Cl_3$ (325.6): C, 54.50%; H, 2.29%; N, 3.97%.
Found: C, 54.40%; H, 2.60%; N, 3.94%.

The ester prepared from this acid was identical with the product described above.

Two grams of pure trichloroisatin was dissolved in 60 ml. of 10% sodium hydroxide and treated with hydrogen peroxide. On neutralization with hydrochloric acid a white solid came out. It weighed 1.92 g. and melted at 226-229°. An analytical sample from aqueous ethanol melted at 229-230.5°.

Anal. Calcd. for $C_7H_4NO_2Cl_3$ (240.5): C, 34.95%; H, 1.68%.
Found: C, 34.98%; H, 1.81%.

The value given in the literature[24] for the melting point of 3,4,5-trichloroanthranilic acid is 226-227°.

One and two-tenths grams of the anthranilic acid was dissolved in 5 ml. of 5% sodium hydroxide. The solution was diluted to 35 ml. and 0.34 g. of sodium nitrite was added. A mixture of ice and 3 ml. of concentrated hydrochloric acid was prepared and the solution was added to it. Then the mixture which contained some pink solid was added to 500 ml. of absolute ethanol containing a little finely ground cuprous chloride. After refluxing for one-half hour the solvent was stripped off and the residue was reprecipitated with acid from solution in ammonia. This gave 0.84 g. of solid melting at 194-207°. An analytical sample melted at 207-210°. The literature[25] gives 203° for the melting point of 3,4,5-trichlorobenzoic acid and 186-187° for 2,3,4-trichlorobenzoic acid.

Anal. Calcd. for $C_7H_3O_2Cl_3$ (225.5): C, 37.27%; H, 1.34%.
Found: C, 37.45%; H, 1.63%.

As a final check the ethyl ester was prepared. It melted at 85-87° while the value of 86° is given in the literature.

The Preparation of 6,7,8-Trichloro-2-phenyl-α-(2-piperidyl)-4-quinolinemethanol Hydrochloride

Although no major alterations of the standard conditions were employed

in the synthesis of this carbinol, the technique of carrying out the reactions required a good deal of experimental investigation before satisfactory results were obtained. The difficulties arose chiefly from the great insolubility of the various intermediates and by-products. They were similar to those encountered by Mr. Rapport in his preparation of the analagous 6,7-dichloro carbinol[7] and his experience with such problems was of great help.

At the time when the preparation of the intermediate bromoketone had been worked out, and one preliminary ring-closure and reduction had been completed, the synthesis was turned over to Dr. A. A. Benson. He completed the preparation of the material for testing; a short summary of his work is appended for the sake of completeness.

The condensation of 6,7,8-trichloro-2-phenylcinchoninic ester and ω-benzamidocaproic ester was carried out as usual. During the hydrolysis with sulfuric acid a large amount of insoluble material precipitated. Following Rapport's method in his dichloro synthesis, this was filtered from the hot sulfuric acid solution and discarded, since extraction of this residue with hot sulfuric acid gave no further product. If this by-product was not removed at this point it interferred with the extraction of the ketone free base.

The working up of the ketone proceeded as usual, except that there was a good deal of material which was insoluble either in sodium hydroxide solution or in chloroform. This was also discarded. On the basis of preliminary experiments it seems likely that this material, and the solid

discarded previously, contained a good deal of 6,7,8-trichloro-2-phenyl-cinchoninic acid; no attempt was made to recover it.

Because of the poor results recorded below on the ring-closure and reduction Mr. Rapport investigated the use of a lower temperature during the ring closure. He obtained about twice as great a yield of final carbinol, and prepared an analytical sample from it. In the hands of Dr. Benson this improvement eventually led to a very satisfactory yield in the last step.

ε-(6,7,8-Trichloro-2-phenylcinchonyl)-n-amylamine hydrobromide. - A solution of 72.7 g. of ethyl 6,7,8-trichloro-2-phenylcinchoninate and 57.8 g. of ethyl ω-benzamidocaproate in 190 ml. of boiling benzene was added to sodamide prepared from 5.4 g. of sodium in the usual way. A vigorous evolution of ammonia commenced at once. The mixture was placed in an oil bath at 100°. After six hours of stirring the initial grey suspension began to turn brown and oily, and after twenty-two hours the whole mass was a brown oil. To this was added 500 ml. of a solution of 300 ml. of concentrated sulfuric acid in 400 ml. of water. The benzene was distilled out of the two phase system, and the residue was refluxed for seventy-two hours.

At this time the hot sulfuric acid solution was filtered free from a substantial quantity of crystalline solid which had formed during the hydrolysis. The filtrate was diluted to 3 l. with ice and water, and brought to neutrality by the addition of solid sodium carbonate. It was then made strongly basic by the addition of 50 ml. of 50% sodium hydroxide solution. After diluting the resulting suspension to 6 l. with water it was carefully extracted with chloroform. Due to the large amount of insoluble, amorphous material which accumulated at the interface this presented some difficulty. A total of 1.5 l. of chloroform was employed in several portions for the extractions. The solid and aqueous residues were discarded.

The combined chloroform extracts were evaporated *in vacuo* leaving a brown tar. To this was added 200 ml. of isopropanol, partially dissolving the tar, and hydrobromic acid (48%) was added until the solution was acid. This caused the conversion of the tar to a brown, crystalline solid, weighing 25.3 g. Recrystallization from 250 ml. of methanol freed the product from a small amount of 6,7,8-trichloro-2-phenylcinchoninic acid (which was insoluble in the hot methanol), and gave a product consisting of light grey platelets, m. p. 196-198° (dec.). The yield of recrystallized product was 19.7 g., 21%. An analytical sample recrystallized from methanol consisted of rosettes of golden-brown bars, m. p. 193-195° (dec.).

Anal. Calcd. for $C_{21}H_{20}N_2OBrCl_3$ (502.7): C, 50.17%; H, 4.01%; N, 5.57%.
Found: C, 50.13%; H, 4.97%; N, 5.48%.

ε-Bromo- ε-(6,7,8-trichloro-2-phenylcinchonyl)-n-amylamine hydrobromide. - To 4.2 g. of ketone hydrobromide dissolved in 50 ml. of 48% hydrobromic acid at 90-95° was added dropwise 1.29 g. of bromine dissolved in 5 ml. of 48% hydrobromic acid. After the addition the solution was kept at this temperature for ten minutes. To the hot solution was added 50 ml. of ethanol, causing a yellow, crystalline solid to precipitate. After cooling this was filtered off, giving 4.2 g. of bromoketone hydrobromide, m. p. 198-199° (dec.), a yield of 86%. An analytical sample crystallized from acetic acid consisted of thick, columnar crystals, m. p. 191-192° (dec.).

Anal. Calcd. for $C_{21}H_{19}N_2OBr_2Cl_3$ (581.6): C, 43.36%; H, 3.29%.
Found: C, 42.74%; H, 3.32%.

6,7,8-Trichloro-2-phenyl-α-(2-piperidyl)-4-quinolinemethanol (SN13150). A preliminary ring-closure and reduction was carried out using the 4.2 g. of bromoketone hydrobromide obtained in the last experiment. Since another method

was eventually evolved for carrying out this reaction it will not be described. There was obtained 200 mg. of crude carbinol hydrochloride, m. p. 250-252° (dec.). An analytical sample was prepared by precipitating the salt from 12 N hydrochloric acid with ethanol.

Anal. Calcd. for $C_{21}H_{20}N_2OCl_4$ (458.2): C, 55.02%; H, 4.40%.

Found: C, 54.60%; H, 4.90%.

(Corrected for 0.4% of ash in the sample.)

By carrying out the ring-closure for a longer period of time at 0° Mr. Rapport increased the yield. He prepared an analytical sample of the free carbinol, m. p. 225-226°.

Anal. Calcd. for $C_{21}H_{19}N_2OCl_3$ (421.8): C, 59.80%; H, 4.54%; N, 6.64%.

Found: C, 59.82%; H, 4.82%; N, 6.62%.

In a large scale run Dr. Benson brominated 26.4 g. of pure ketone hydrobromide, and converted the bromoketone thus obtained to the carbinol following Rapport's method of ring-closure. He thus obtained 10.7 g. of carbinol hydrobromide, which gave 6.32 g. of free base, identical with Mr. Rapport's analytical sample. This is a yield of 29% of crude carbinol from the ketone hydrobromide. This carbinol was combined with the products of several preliminary preparations, purified, and submitted for testing as the hydrochloride.

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V. INVESTIGATION OF THE EFFECT OF GLOBULIN DEPLETION ON ANTIBODY PRODUCTION IN RABBITS

A. INTRODUCTION

It is now generally accepted that antibodies are associated with a specific protein fraction of the blood serum known as γ-globulin. Numerous explanations have been advanced to explain the appearance of these antibodies soon after the injection into the animal of the corresponding antigen.

According to the theory advanced by Pauling (1) the presence of an antigen at the site or sites of γ-globulin synthesis causes the long polypeptide chains to fold in such a manner that they assume configurations complementary to the antigen, thus acquiring the ability to combine with it. If the production of γ-globulin is a normal activity of the body, and not a specific response to the presence of antigen, the theory suggests that, in the presence of a certain amount of antigen, an increased rate of γ-globulin production should lead to a higher proportion of antibody molecules in the serum. This follows from the assumption that only a fraction of the circulating γ-globulin of the serum will be replaced during the effective presence of the antigen in the body, so that only a fraction of the globulin will be specific for that antigen.

The method we proposed to apply to stimulate an increased rate of γ-globulin formation was a severe depletion of the circulating globulins brought about by bleeding the animals at frequent intervals and

reinjecting their erythrocytes suspended in an amount of isotonic homologous serum albumin sufficient to replace the volume of serum lost. A somewhat similar experiment has been reported by Cannon and his co-workers (2) who brought about the desired depletion by prolonged feeding of the experimental animals with a protein-free diet. In his experiments no positive conclusions could be drawn concerning the effect of this treatment on the production of antibodies; immunization was attempted either during the starvation period or following it when the animal was being fed a diet rich in proteins, and hence was presumably synthesizing proteins rapidly.

The experiments described here are of a preliminary nature and failed to demonstrate whether it would actually be possible to observe the postulated effect. Several unexpected difficulties were encountered during the investigation and it now appears that a much more refined technique will be required to find the answer. The experimental section which follows describes what has been done, while some suggestions for further work are embodied in the discussion following.

B. EXPERIMENTAL

A. First Experiment

Depletion Technique.- Thirteen young rabbits, ranging in weight from 2.1 to 2.8 kg., were separated arbitrarily into a group of six controls and a group of seven test animals. Approximately 30 ml. of blood was taken from each test animal every 48 hours. The first two bleedings were from the marginal ear vein into paraffined 50 ml. centrifuge tubes, while the remainder were by heart puncture with a No. 20 gauge needle; in every case 2 ml. of 10% sodium citrate was present in the centrifuge tube or syringe to prevent clotting.

The cells from each rabbit were centrifuged down and washed three times with sterile 0.9% saline solution. The cells were then resuspended in sufficient 3% rabbit albumin solution, containing 0.9% sodium chloride, to bring the volume of the suspension up to that of the whole blood originally taken from the animal. The suspension was filtered through sterile gauze and reinjected by marginal ear vein into the same rabbit from which the cells came originally. The entire process of bleeding, washing, and reinjecting required 2 to 3 hours.

Analyses for Serum Albumin and Globulin.- To 1 ml. of citrated plasma was added 0.35 ml. of 2% calcium chloride solution; the serum was then diluted to 5 ml. with 0.9% sodium chloride solution and allowed to clot at 37°. The liquid was expressed from the clot and the fibrin was then removed and discarded. 4.6 ml. of saturated ammonium sulfate

was then added and the solution was kept at room temperature for twenty to thirty minutes before centrifuging down the globulin. The globulin precipitate was dissolved in saline and suitable aliquots of it and the albumin supernatant were taken for analysis by a modified Folin microcolorimetric method (3). Since no calibration factor for albumin was available, both albumin and globulin values are reported as rabbit serum globulin.

Immunological Techniques.- At the end of the depletion period (5 to 8 bleedings per rabbit) each rabbit was injected intraperitoneally with 100 mg. of ovalbumin in 5 ml. of isotonic saline. The immunizing injection was repeated after seven days. Ten days after the first injection preliminary ring tests with 1% ovalbumin and undiluted serum showed positive results in all but one of the four surviving rabbits and all but one of the six controls. Accordingly, three days later 15 ml. of blood was taken from the ear of each rabbit and the undiluted sera titrated, with the results in Table II. After eight days the rabbits were again bled and their sera titrated; the results are given in Table III.

B. Second Experiment

Preparation of 3% Albumin Solution.- Rabbit serum was diluted with an equal volume of saturated ammonium sulfate and the precipitate filtered off. The supernatant was dialyzed against running tap water for 2 to 3 days and then concentrated to the original volume of the serum by evaporation in the dialyzing membranes, which were suspended

Table I

Rabbit No.	Weight in kg.		Albumin / Globulin Ratio in Serum*							
	Bleeding		Bleeding							
	1st	8th	1st	2nd	3rd	4th	5th	6th	7th	8th
200	2.2	2.0	419/183	387/154	220/165	342/144	209/122	302/129	244/168	247/218
202	2.6	2.3	494/173	512/178	419/166	454/96	334/57	485/103	358/84	407/193
203	2.7	2.6	—	399**/162	430/116	478/108	272/70	485/122	202/129	411/84
205	2.4	2.1	476/132	426/94	403/139	354/129	147/96	298/130	195/151	258/141
207	2.8	2.6	—	—	**	446/56	379/64	383/144	399/36	391/71

* Numerator is μg. of albumin per ml. of serum, expressed as serum globulin. Denominator is μg. of globulin per ml. of serum.

** First bleeding for this rabbit.

Table II

Titration of Antiovalbumin Sera

	Rabbit No.	Ovalbumin Dilution 1:5000	1:7500	1:11,250	1:16,875	1:25,313	1:39,970	Saline control
Depleted	202	+	+	+	+	++	−	−
	203	+	+	++	+	+	+	−
	205	−	−	−	−	−	−	−
	207	+	+	+	+	+	+	+
Control	204	−	−	−	−	−	−	−
	206	+	+	+	+	+	+	+
	213	−	−	−	−	−	−	−
	215	+	+	+	+	+	++	+
	216	+	+	+	+	+	++	+
	217	+	+	+	+	+	++	+

Test was read visually after 20 to 30 minutes at 37°.

Table III

Titration of Antiovalbumin Sera*

	Rabbit No.	Antigen Dilution 1:20,000	1:30,000	1:45,000	1:67,500	1:101,250	1:151,875	1:227,813	Saline Control
Depleted	202	400	460	650	870 max.	(1000)			133
	203	212	216	220	246	280	315	(max?)	186
	205	190	204	178	183	180	165		254
	207	246	310	270	325	---	365 max.	356	435
Control	206	202	165	169	244	--- max.	155	236	690
	213	248	345	270	315	---	400	475	115
	215	152	152	160 max.	128	148			166
	216	94	240	320 max?	400 max?	550	600		---
	217	165	280	520	570 max.	---	900	1300	445

* The figures are colorimetric readings representing relative amounts of precipitate. The ind icated maxima were estimated visually one-half hour after mixing.

in a current of warm air. The solution was sterilized by passing through a Seitz filter and stored at 5°.

When an attempt was made to increase the severity of the depletion conditions by increasing the amount of each bleeding from 30 ml. to 50 ml. and by decreasing the interval between bleedings from 48 hours to 24 hours, seven of the ten test animals died within 24 hours. The difficulty was traced to the albumin solution, which was found to be toxic when 30 ml. of the 3% solution was injected into each of three rabbits.

C. Third Experiment

More albumin was prepared as before, but the solution, instead of being stored after sterilization, was lyophilized. A sterile 3% solution was prepared in isotonic saline.

The test animals were bled 50 ml. each from the heart for four consecutive days. At the first bleeding 40 to 50 ml. of 3% albumin was injected into each rabbit immediately after bleeding. After the second bleeding 50 ml. of a suspension of erythrocytes, made up of two volumes of pooled, washed cells in three volumes of albumin solution, was injected, and after the third bleeding 40 ml. of albumin solution was administered. The hematocrit fell rapidly during the depletion experiments and was approximately 20% at the fourth day. It was intended to bleed only four times and inject the first immunizing dose of ovalbumin on the fifth day; however, only one rabbit survived the fourth bleeding and it died three days after the first ovalbumin injection.

C. DISCUSSION

In the first experiment we had hoped to be able to drop the globulin content of the blood of the rabbits employed to a low level during the course of a short series of bleedings, while re-injecting, in so far as possible, the other components of the blood in order to keep disturbing physiological complications to a minimum. Although the majority of the animals survived, Table I shows that no significant increase in the ratio of albumin to globulin can be noted in the bled animals during the course of the experiment, nor can any significant trend be noted in the absolute amounts of globulin in the serum.

Although no decrease in the amount of globulin in circulation had been shown by analysis of the fractionated sera it was felt that, since a large quantity of globulin had been removed in the course of two weeks, it was probable that the rabbits would be synthesizing globulins at an increased rate. Therefore we decided to proceed with the immunization in the hope of observing some significant difference between the response of the bled animals and the controls. Tables II and III show the results of experiments on the immune sera so obtained. On the basis of this evidence no conclusions concerning differences in the antibody titers of the two sets of rabbits can be drawn.

Due to this failure an attempt was made to use a much stronger program of depletion. It proved to be too strenuous for the animals to survive, so no tests could be run on immunization. In all probability

it was the lack of a sufficient supply of red cells, combined with the general severe treatment which caused the death of the rabbits.

At this point the experiments were dropped. In suggesting future paths which this research should take it appears to the authors that the whole problem of the depletion technique should be investigated more thoroughly and carefully. It will be impossible to draw any conclusions from the results of immunization until there is at hand a group of rabbits which has been depleted so thoroughly that a definite and easily demonstrable drop in the globulin content of the serum is obtained. This will probably require a very strenuous program of bleeding and reinjection, with special emphasis on the maintainance of the hematocrit at a point sufficient to sustain life.

Acknowledgment

This investigation was carried out in collaboration with Mr. J. W. Sease. The authors wish to thank Professor Dan H. Campbell, who suggested the problem and directed the work, Mr. George Feigen, who performed the heart punctures and gave many helpful suggestions, and Mary L. Sease, who assisted with the experimental work.

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SUMMARY

I. A brief discussion is given of the reason for expecting antimalarial activity in analogues of pantothenic acid, based on the Fildes-Wood theory of the mode of action of sulfonamides. As part of a program for testing this hypothesis, the syntheses of seven such analogues were carried out. These were γ-hydroxybutyryltauryl-2-aminopyrimidine, pantoyltauryl-2-aminopyrimidine, pantoyl-β-aminoethylphenylsulfide, pantoyl-β-aminoethylphenylsulfoxide, pantoyl-β-aminoethylphenylsulfone, pantoyl-β-aminoethyl-p-chlorophenylsulfide, and pantoyl-β-aminoethyl-p-chlorosulfone.

II. The preparations are described of two derivatives of sulfonanilamide, N^1-(3,5-dibromo-4-aminophenyl)-sulfanilamide and N^1-(3,5-dibromo-4-methylaminophenyl)-sulfanilamide. Although they are devoid of antimalarial activity, they are of pharmacological interest, since their bacteriostatic action is not antagonized by p-aminobenzoic acid, in contrast to most drugs of the sulfanilamide class.

III. A number of attempts to prepare derivatives of sulfadiazine brominated in the four ron six positions of the pyrimidine ring are reported. Attempts to prepare these compounds by the reaction of brominated 2-aminopyrimidines with sulfonylchlorides failed due to the low basicity of the 2-amino group in such compounds, while attempts to brominate hydroxysulfadiazines were unsuccessful. Although it was possible to prepare p-nitrobenzenesulfonamido-2-amino-4-bromo-6-methylpyrimidine all attempts to reduce the nitro group to an amino group

failed. The synthesis was eventually abandoned.

In the course of this research an interesting decomposition reaction of p-nitrobenzenesulfonamidopyrimidines was discovered in which the SO_2 group was split out. Preliminary experiments on the nature of this reaction are described.

IV. There are described the preparations of four compounds of the "Ainley-King" type, 2-cyclohexyl- α-(2-piperidyl)-4-quinolinemethanol, 7-chloro- α-(2-piperidyl)-4-quinolinemethanol, 7-chloro-2-phenyl- α-(2-piperidyl)-4-quinolinemethanol, and 6,7,8-trichloro-2-phenyl- α-(2-piperidyl)-4-quinolinemethanol. In addition the failure to synthesize 5-chloro- α-(2-piperidyl)-4-quinolinemethanol and 5-chloro-2-phenyl- α-(2-piperidyl)-4-quinolinemethanol is reported. These compounds were prepared using the Sargent modification of the procedure originally devised by Ainley and King. In the course of the work the preparation of several new derivatives of isatin and cinchoninic acid were worked out.

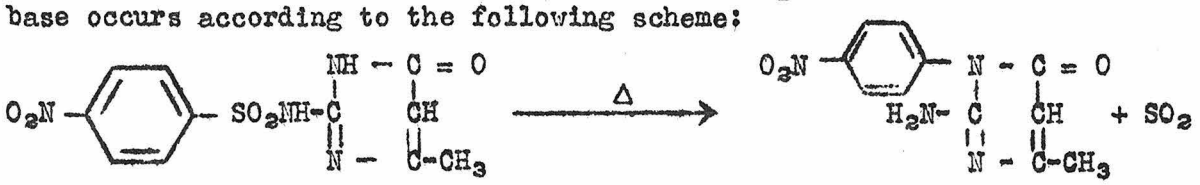
V. An attempt was made to investigate the rate of antibody production in rabbits, after they had been severly depleted of serum globulin. In the preliminary experiments recorded, it was not possible to find a method of protein depletion which would permit the gathering of significant data. In the first experiment it was impossible to demonstrate any significant decrease in the amount of globulin in the plasma during the experiment, in latter experiments with heavier bleedings the rabbits failed to survive.

Propositions Submitted by Allen E. Senear

Ph.D. Oral Examination, August 16, 1946; 9:00 A.M.

Committee: Dr. Koepfli (Chairman), Professors Pauling, Zeichmeister, Lucas, Badger, Niemann, Campbell, Schomaker and Dr. Buchman.

1. It is proposed that the decomposition of 2-(p-nitrobenzenesulfonamido)-4-methyl-6-hydroxypyrimidine upon heating or treatment with base occurs according to the following scheme:



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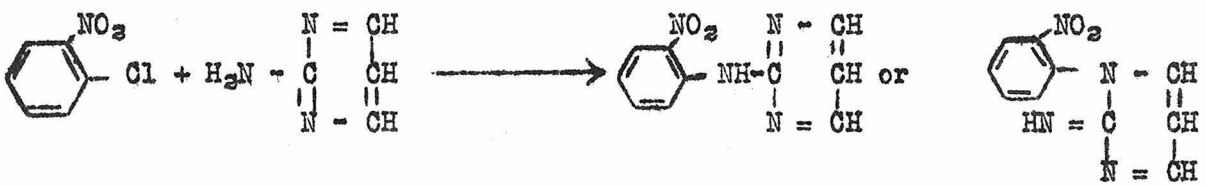
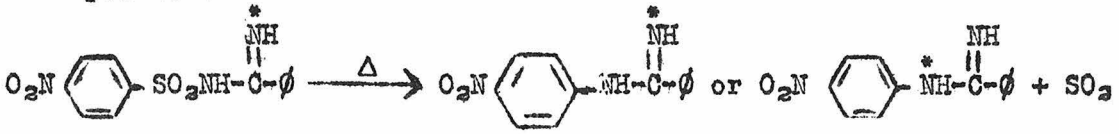
2. Two decompositions similar to that discussed in 1 have been reported in the literature. It seems probable that upon close examination they may also be found to involve a shift of a nitrophenyl group.

a. The conclusion of English and his co-workers that the decomposition of 2-(O-nitrobenzenesulfonamido)-pyrimidine led to 2-(O-nitroanilino)-pyrimidine is not proved. The reaction of O-nitrochlorobenzene and 2-aminopyrimidine might well lead to 1-(O-nitrophenyl)-2-iminopyrimidine:



b. In the case of the reaction reported by Barber, it should prove easy to determine the nature of the reaction using N^{15} and tracer technique, even though ordinary chemical methods will not resolve this problem:

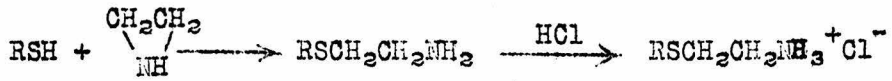


English et al, J. A. C. S. 68, 1039 (1946).

Barber, J.C.S. 101 (1943).

3. The use of ethylenimine as a reagent in organic chemistry should be extended.

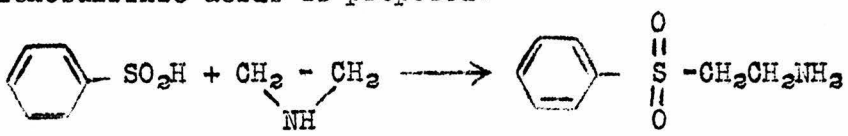
a. The preparation of β-aminoethylsulfides from ethylenimine and mercaptans appears to be a general reaction. The amine hydrochlorides obtained in suitable cases may serve as derivatives for characterization:



This Thesis: Pg. 19

C.A. P3263[8] (1943)

b. The extension of this reaction to the direct preparation of β-aminoethylphenylsulfones, by the interaction of ethylenimine and benzenesulfinic acids is proposed:



Gabriel, Ber. 21, 2667 (1888)

Goldberg, J.C.S. 826 (1945).

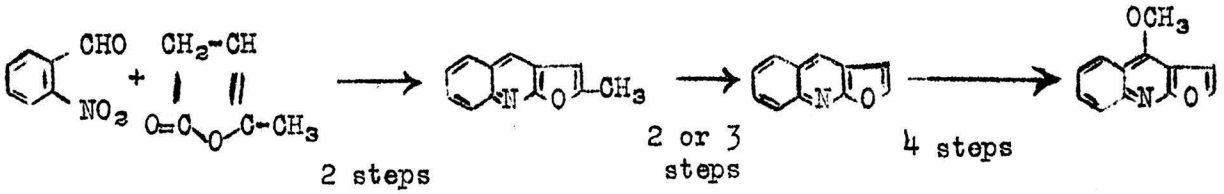
4. It is proposed that the fractional precipitation of isatin derivatives substituted in the 4- and 6-positions may be understood by considering the stabilizing effect of hydrogen bonding in isatinic acids, and the interference of 4-substituents with this stabilization.

Thesis: Pg. 99

von Braun et al: Ann. 451 26 (1926)

Pauling: "The Nature of the Chemical Bond", Section 41

5. The synthesis of alkaloids of the furnanoquinoline type, such as dictamnine, has not been carried out. A method of attack on this problem, via the intermediates shown, is proposed:

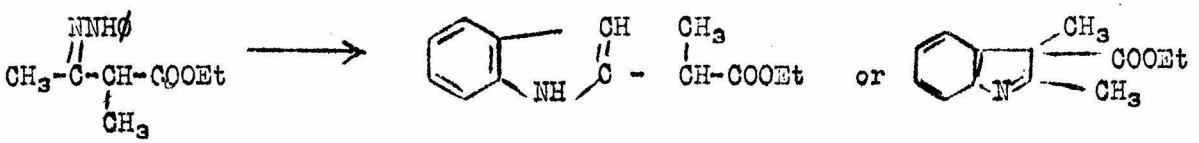


Henry: "The Plant Alkaloids". Pg. 390

Haq, Kapur, and Ray, J.C.S. 1087 (1933)

Bergstrom, Chem. Rev. 35, 77(1944)

6. The assumption by Walker that the compound obtained when ethyl-α-methylacetoacetate phenylhydrazone undergoes the Fisher indole reaction can only be the ethyl ester of α-methylindole-2-acetic acid is in error. Not only is an indolenine structure possible, but on the basis of the accepted mechanism of this reaction it would seem to be more likely.



Walker, Am. Chem. J. 16, 430 (1894)

Robinson and Robinson, J. C. S. 125, 827 (1924).

7. a. It has been suggested that pantothenic acid is of enhanced importance in the metabolism of bacteria which have developed "fastness" to sulfanilamide. This indicates that drugs of the pantoyltaurine type may be of value in the treatment of sulfanilamide resistant cases. The development of an alternation technique in the chemotherapy of bacterial infections should prove of value.

Sevag and Green, J. Bact. 48, 631 (1944).

b. Recent publications have disclosed a number of drugs structurally related to sulfanilamide, which differ from the latter in their mode of action. The study of the interaction of such drugs with sulfanilamide-fast bacteria should provide important information concerning the nature of the action, and the nature of sulfanilamide fastness.

English et al, J.A.C.S. 68, 453, 939 (1946).

Lawrence and Goetchius, J. Bact. 49, 575 (1945).

Marshal, Litchfield, and White, J. Pharm. 86, 273 (1946).

Senear, Head, Rapport, Maynard, and Koepfli, J. O. C., to be published.

8. a. It is proposed that the reduction of the ketone of the 6,8-dichloro-2-(p-chlorophenyl)-Ainley King compound be carried out using Al $(i\text{-}PrO)_3$, in an attempt to elucidate the nature of the persistent impurity which makes pyrification of this compound so difficult.

Ref. Unpublished information, obtained by Dr. A. A. Benson during the preparation of this compound.

b. I propose that the carbobenzoxy group may offer advantages over the benzoyl group, for the usual Ainley-King synthesis.

9. The ability to describe crystals correctly is important to organic chemists; yet to many of them such descriptions merely offer opportunities to wax lyrical. I therefore propose

a. That some chemist, preferably one familiar with the limitations of organic chemists, correlate the available information on types of crystals, and devise a suitable nomenclature for their rapid description.

b. That the assimilation of this information, and training in its use, be part of the undergraduate training of chemists.