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Thesis by

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The Synthesis of Potential Antimalarials *

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This section is concerned with the preparation of two series of potential antimalarial drugs.

The importance of the malaria problem has long been appreciated, and the search for new chemotherapeutic agents has, accordingly, occupied the attention of many investigators. The war gave new impetus to the search because of extensive military operations in tropical regions, and a large program under the auspices of the National Research Council was initiated to survey new, potentially active antimalarial drugs.

Initially, efforts were directed to finding a substitute for quinine which is useful in both suppressive therapy and in treating the acute attack of malaria. Clarification of the pharmacology of quinacrine (Atabrine) established this drug as equal, and in some respects superior, to quinine in effectiveness. There followed a shift in emphasis to the following two objectives: first, a drug which will act as a causal prophylactic, i.e., one which prevents sporozoite infection; and second, one which will cure relapsing vivax, the most important chronic form of human malaria.

Determining the antimalarial activity of new compounds presents a large number of problems. Because it is not practical to test new drugs directly on malarial infection

in man, they are first screened on avian malarias, usually against Plasmodium lophurae in ducks and Plasmodium gallinaceum in chicks. These screening tests are of little value, however, in determining the effect of a drug on the human malarias because of the biological complex encountered in host specificity and species and strain susceptibilities. Moreover, the course of malaria in humans and in birds may differ considerably. In the cycle of P. gallinaceum in chick malaria, an exoerythrocytic stage (tissue form) has been positively identified; in human infections, no such stage has been detectable, although the existence of such forms is postulated in one attempt to explain relapsing after cure.

The results of the screening tests in birds determine whether a compound is to be dismissed from further investigation or subjected to more intensive study, but the final evaluation must rest upon the outcome of clinical trial in humans. This difficulty in determining activity is, at the present time, the slow step in the search for new chemotherapeutic agents.

Carbinolamines Related to Quinine

The first successful synthesis of a simple compound patterned on the quinine molecule and showing antiplasmodial activity in avian malaria was reported by Ainley and King (1) in 1938. The compound was (6-methoxyquinolyl-4)-α-piperidylcarbinol. Kelsey, Geiling, Oldham, and Dearborn (2) in 1943 isolated a product obtained by the in vitro action of rabbit liver on quinine, and Mead and Koepfli (3) presented evidence in 1944 that this metabolic derivative was 2'-hydroxy-6'-methoxy-3-vinylruban-9-ol. This structure differed from quinine only in the substitution of an OH group for H in the 2-position of the quinoline nucleus, and this position was therefore indicated as the site of primary attack in the degradation of the quinine molecule. The marked dependence of the therapeutic activity upon the rate of degradation with this type of drug was emphasized by the observation that cinchonine was much more active than quinine on a basis of blood level but somewhat less active at a given oral dosage (4), and that this phenomenon was due presumably to the greater rate at which cinchonine was broken down.

Accordingly, it was thought that an increase in the antiplasmodial action of quinine-like drugs could be effected by blocking this 2-position, and the work to be described here, which was begun in April of 1944, is con-

cerned with compounds having the same pattern as that reported by Ainley and King but containing a phenyl group in the 2-position of the quinoline nucleus. A similarly substituted dihydrocinchonine is also described.

This modification was uncommonly successful and resulted in greatly increased activity in the quinolylpiperidylcarbinols. As an example, the very first compound of this type to be synthesized, (2-phenylquinolyl-4)-α-piperidylcarbinol was 48 times as effective as the 2-unsubstituted analog and 3 times as active as quinine against P. lophurae in ducks.

The method of synthesis employed was that described by Ainley and King (1) with the improvements effected by Sargent and Buchman (5). It is outlined on the following pages.

In this synthesis, the ethyl ester of an appropriate cinchoninic acid (A) is condensed with ethyl ε-benzamidocaproate (B) to give a quinolyl-4 α-carbethoxy-ε-benzamidoamyl ketone (C). This ester is then hydrolyzed and the resulting acid decarboxylated to give a quinolyl-4 ε-benzamidoamyl ketone (D) which upon further hydrolysis is converted to a quinolyl-4 ε-aminoamyl ketone (E). Bromination gives a quinolyl-4 α-bromo-ε-aminoamyl ketone (F). The ring is then closed with aqueous sodium carbonate, and the

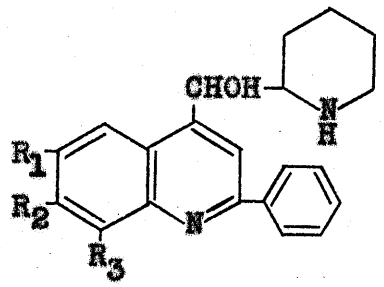
resulting quinolyl-4 α-piperidyl ketone (G) is catalytically reduced to the quinolyl-4-α-piperidylcarbinol (H).

The intermediates C, D, and G are not isolated, while E may be isolated when the circumstances make it advisable. Generally, the only intermediate stage at which isolation and purification are effected is F, the bromoketone. In the 2-phenyl series, the intermediates appeared to be more stable than those in the original Ainley and King synthesis, and it was not necessary to employ many of the precautions suggested by them and by Sargent and Buchman.

The 2-phenylcinchoninic acids required for this synthesis are most readily obtained by two methods. In the Pfitzinger procedure (6) an appropriately substituted isatin is condensed with acetophenone in alcoholic potassium hydroxide. In the Doebner method (7) pyruvic acid is condensed with benzaldehyde and a substituted aniline in ethanol. Both methods were employed; although the Doebner method is simpler and more direct, it is less general than the Pfitzinger reaction.

The other starting material, ethyl ε-benzamidocaproate, was obtained from Dr. C. C. Price of the University of Illinois.

The compounds which were prepared in this series are listed on the following page.



I (2-Phenylquinolyl-4)-α-piperidylcarbinol

R_1 -H R_2 -H R_3 -H

II (6-Methoxy-2-phenylquinolyl-4)-α-piperidylcarbinol

R_1 $-OCH_3$ R_2 -H R_3 -H

III (8-Methoxy-2-phenylquinolyl-4)-α-piperidylcarbinol

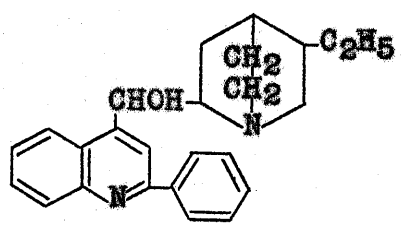
R_1 -H R_2 -H R_3 $-OCH_3$

IV (8-Hydroxy-2-phenylquinolyl-4)-α-piperidylcarbinol

R_1 -H R_2 -H R_3 -OH

V (6,7-Dichloro-2-phenylquinolyl-4)-α-piperidylcarbinol

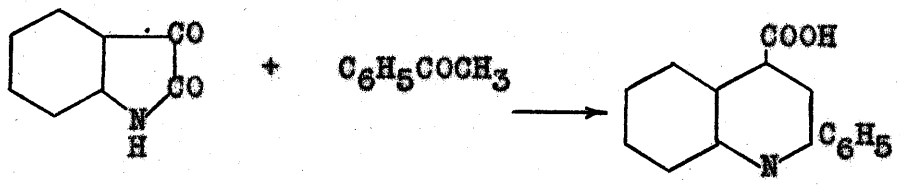
R_1 -Cl R_2 -Cl R_3 -H

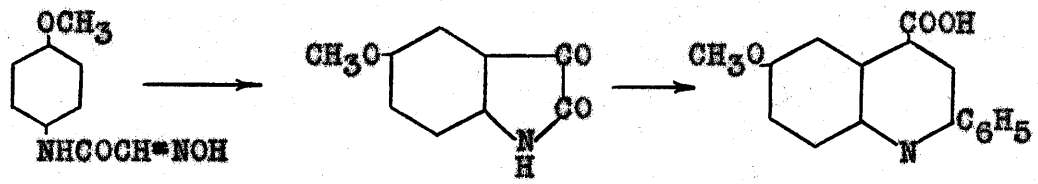


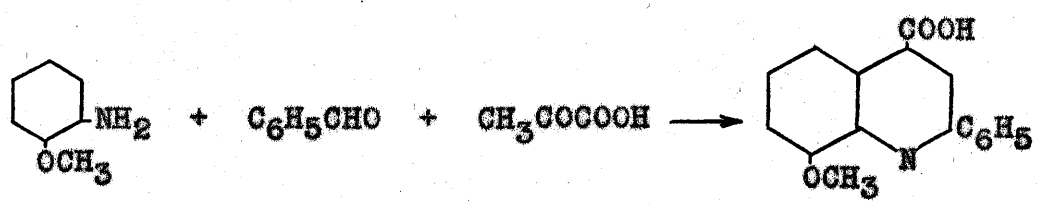
VI 3-Ethyl-2'-phenylruban-9-ol

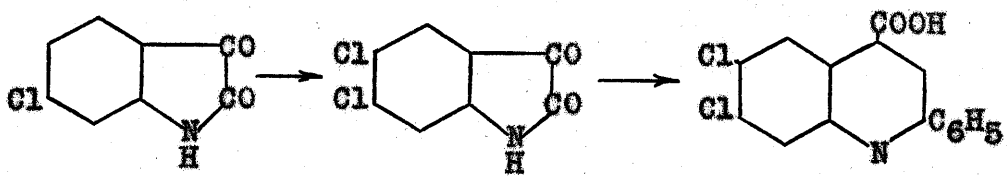
"2-Phenyldihydrocinchonine"

Synthesis of Substituted 2-Phenylcinchoninic Acids

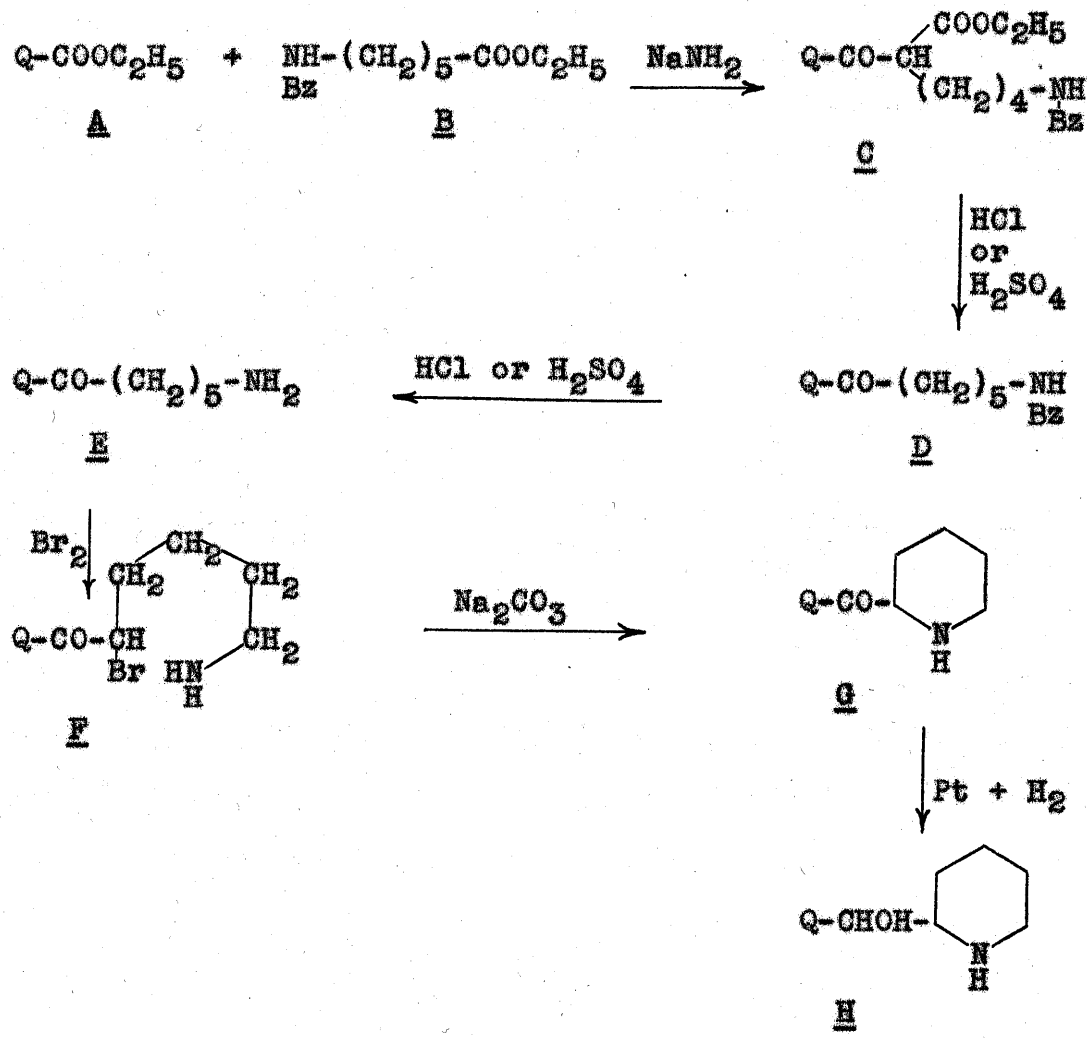
I 

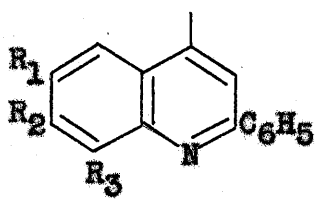
II 

III 

IV 

Synthesis of Quinolyl-4-α-Piperidylcarbinols



where Q is 

and Bz is C_6H_5CO (benzoyl)

(2-Phenylquinolyl-4)-α-Piperidylcarbinol I

2-Phenylcinchoninic acid was prepared from isatin and acetophenone in quantitative yield according to the directions of Pfitzinger (6). The acid was then esterified in 77 per cent yield by the procedure employed by Ainley and King (1) to esterify cinchoninic acid.

Ethyl 2-phenylcinchoninate was then condensed with ε-benzamidocaproate in benzene using sodamide as the condensing agent, and the product was hydrolyzed with 6N hydrochloric acid.

It was unnecessary to isolate the ketone, although the latter was readily obtainable as the crystalline hydrobromide. The hydrobromic acid extract of the ketone was brominated using a solution of bromine in hydrobromic acid, and the crystalline bromoketone dihydrobromide was obtained in 52 per cent yield based upon the quantities of esters which were condensed.

The piperidine ring was closed in aqueous ethanol with sodium carbonate, and the resulting piperidyl ketone was immediately reduced with platinum oxide and hydrogen to give a 70 per cent yield of the quinolylpiperidylcarbinol isolated as the dihydrochloride.

Experimental Part[1]

2-Phenylcinchoninic acid (6). From 60 g. of isatin, 90 g.

[1] Melting points are corrected.

of acetophenone, 480 ml. of absolute ethanol, and 240 ml. of 33 wt.% potassium hydroxide, 103.5 g. of crude cinchophen were obtained following the procedure of Pfitzinger. The first precipitation of the acid gave a reasonably pure product, and it was unnecessary to employ the elaborate purification he described. The yield was quantitative, and the dry crude product was esterified directly.

Ethyl 2-phenylcinchoninate (6). To a suspension of 102 g. (0.41 mole) of cinchophen in 500 ml. of absolute ethanol, 50 ml. of conc. sulfuric acid were added. The mixture was refluxed for 7 hrs., a clear solution being obtained in 45 min. The ethanol was removed under reduced pressure, and the residual oil was poured into a mixture of 1 kg. of ice and 500 ml. of water. The mixture was brought to pH 9 with conc. ammonia solution (130 ml.), and the ester was extracted with 400, 250, and 150 ml. portions of ether. After drying over sodium sulfate, the ether was removed, and the residual oil was distilled, b.p. 186-191°/1.5 mm., to give 87 g. (77%).

(2-Phenylquinolyl-4) α-bromo-ϵ-aminoamyl ketone dihydrobromide. Sodamide was prepared from 38 g. (1.65 mole) of sodium according to the directions of Sargent and Buchman (5).

To the powdered sodamide in a 3-necked, 5 l. flask equipped with a Hirshberg stirrer and a condenser protected

with a soda-lime tube, a solution of 360 g. (1.30 mole) of ethyl 2-phenylcinchoninate[2] and 345 g. (1.31 mole) of ethyl ∈-benzamidocaproate in 675 ml. of dry benzene was added. An oil bath was placed under the flask and stirring was begun with a powerful motor. The bath was kept at 90° for 22 hrs., stirring being continued throughout this period. The bath was then removed.

The reaction mixture was cooled in an ice water bath while a solution of 1200 ml. of conc. hydrochloric acid and 1000 ml. of water was added. The stirrer was then replaced with a stillhead, and the benzene was steam distilled until the temperature of the vapors reached 108°. After substituting a condenser for the stillhead, refluxing was continued for 40 hrs. The solution was then cooled and made alkaline (pH 10-12) with 50 wt.% sodium hydroxide solution (920 ml.). The ketone was extracted with 1500 ml. of chloroform in several portions. After drying over sodium sulfate the chloroform layer was extracted with 750 g. of 40% hydrobromic acid. The chloroform was removed from the hydrobromic acid layer by heating it in a hot water bath and stirring for 30 min. The increase in weight of the solution was then found to be 304 g.

Upon chilling, crystals of (2-phenylquinolyl-4) ∈-aminoamyl ketone dihydrobromide separated out. A small sam-

[2] Obtained from Dr. R. C. Elderfield of Columbia University.

ple of this ketone salt was isolated. After two recrystallizations from 96% ethanol, it was obtained as yellow clusters of needles, m.p. 225-227° (decomp.) at 0.8°/min. from 209°.

$C_{21}H_{22}ON_2 \cdot 2HBr$ (480.3)	Calc.	C	52.5	H	5.0	N	5.8
	Found	C	52.8	H	5.1	N	5.7

The hydrobromic acid suspension of the ketone was heated to 85°, and, with mechanical stirring, a solution of 138 g. of bromine (90% of the theory required by the increase in weight) in 275 ml. of 40% hydrobromic acid was added over a 20 min. period. The temperature was kept at 85-90°; the product began to crystallize out before all the bromine was added. The mixture was heated to the boiling point and 250 ml. of 40% hydrobromic acid were added, but the product did not dissolve. The reaction mixture was chilled, and the product crystallized as a solid mass. It was collected on a sintered glass funnel, washed by suspension in i-propanol to remove hydrobromic acid, then with acetone until the filtrate was colorless, and finally with ether. After drying over sodium hydroxide, 334.5 g. of a light yellow powder were obtained, m.p. 210.5-212° (decomp.) at 1°/min. from 180°. An additional crop of 41 g., m.p. 210-211°, was obtained by concentration of the mother liquors to half the volume. The total yield was 375 g. (52%).

A sample recrystallized for analysis from methanol-i-propyl ether was obtained as yellow prisms, m.p. 197-198° (decomp.) at 1°/min. from 180°.

$C_{21}H_{21}ON_2Br.2HBr$	Calc.	C	45.1	H	4.2	N 5.0
(559.2)	Found	C	45.1	H	4.4	N 4.9

By acidifying the aqueous phase from the chloroform extraction of the ketone and washing the precipitate with ethanol to remove benzoic acid, 96 g. of crude cinchophen were recovered.

(2-Phenylquinolyl-4)-α-piperidylcarbinol dihydrochloride. To a suspension of 140 g. (0.25 mole) of bromoketone salt in 2200 ml. of absolute ethanol in a 4 l. bottle, 735 ml. of 15 wt.% sodium carbonate solution were added. After displacing the air with nitrogen, the bottle was stoppered and shaken mechanically for 50 min. 3.0 g. of platinum oxide were then added, and the bottle was filled with hydrogen. The reduction, at room temperature under atmospheric pressure of hydrogen, was allowed to proceed for 4 hrs., at which time the rate of hydrogen absorption had fallen from 100 ml./min. initially to 1 ml./min. The total uptake was 6.7 liters; theory required 7.1 liters.

The catalyst and precipitated salts were filtered off, and the ethanol was removed under reduced pressure. After decanting the aqueous phase, the residual oil was rinsed

with water and dissolved in 1000 ml. of absolute ethanol. This solution was filtered and 50 ml. of conc. hydrochloric acid were added. The precipitate was filtered, washed with acetone, and dried to give 71 g. of a light pink powder, m.p. 226-228° (decomp.). Recrystallization was effected as follows. 25 g. of crude product were dissolved in 190 ml. of boiling water containing 3 ml. of conc. hydrochloric acid. 1150 ml. of acetone were added, and the solution was chilled overnight to give 18.2 g. of pure, almost colorless prisms of dihydrochloride hemihydrate, m.p. 230-230.5° (decomp.) at 0.6°/min. from 210°.

$C_{21}H_{22}ON_2 \cdot 2HCl \cdot \frac{1}{2}H_2O$	Calc.	C	63.0	H	6.3	N 7.0
(400.4)	Found	C	62.8	H	6.8	N 6.9

From 86% ethanol the compound crystallized as stout, colorless prisms of the monohydrochloride monohydrate, m.p. 221-222° (decomp.) at 0.5°/min. from 210°.

$C_{21}H_{22}ON_2 \cdot HCl \cdot H_2O$	Calc.	C	67.6	H	6.8	N 7.5
(372.9)	Found	C	67.7	H	6.4	N 7.3

The free base was obtained by neutralizing an alcoholic suspension of the dihydrochloride with 5 N sodium hydroxide, removing the solvent, and crystallizing the dry residue from i-propyl ether. The crystals were either colorless needles or flat prisms, m.p. 141.5-143° at 0.7°/min. from 120°.

$C_{21}H_{22}ON_2$	Calc.	C	79.2	H	7.0	N	8.8
(318.4)	Found	C	79.4	H	7.2	N	8.9

From methanol the compound crystallized beautifully as colorless long prisms containing one molecule of solvent and melting with effervescence at 91-94° at 1°/min. from 75°.

$C_{21}H_{22}ON_2.CH_3OH$	Calc.	C	75.4	H	7.5	N	8.0
(350.4)	Found	C	75.3	H	7.3	N	8.2

(6-Methoxy-2-Phenylquinolyl-4)-α-Piperidylcarbinol II

2-Phenylquininic acid was prepared from acetophenone and 5-methoxyisatin by the Pfitzinger reaction. It had been previously prepared by Doebner (7) in about half the yield (from p-anisidine) obtained by the method used here. The acid was converted to the ester in 73 per cent yield with ethanol and sulfuric acid.

Ethyl 2-phenylquininate was condensed with ethyl benzamidocaproate in benzene using sodamide as the condensing agent, and the product was hydrolyzed with 6 N hydrochloric acid.

The hydrobromic acid extract of the aminoketone was

treated with a solution of bromine in hydrobromic acid to give a 23 per cent yield of crystalline bromoaminoketone dihydrobromide.

The piperidine ring was closed in aqueous ethanol with sodium carbonate, and the reduction to the quinolylpiperidylcarbinol was then carried out with platinum oxide and hydrogen to give a 68 per cent yield isolated as the dihydrochloride.

Experimental Part[1]

5-Methoxyisatin (8). From 100 g. of p-methoxyisonitrosoacetanilide[2], 42 g. (46%) of 5-methoxyisatin were obtained according to the directions of Golding (8).

2-Phenylquininic acid (7). From 22 g. (0.125 mole) of 5-methoxyisatin, 75 ml. of 33 wt.% potassium hydroxide, 150 ml. of absolute ethanol, and 28 g. (0.234 mole) of acetophenone, 34.2 g. (98%) of crude 2-phenylquininic acid, m.p. 236-239° at 1.5°/min., were obtained following the procedure described for the preparation of 2-phenylcinchoninic acid.

Ethyl 2-phenylquininate (9). From 34.5 g. (0.123 mole) of crude 2-phenylquininic acid, 175 ml. of absolute ethanol, and 18 ml. of conc. sulfuric acid, 27.8 g. (73%) of ethyl 2-phenylquininate were obtained following the procedure de-

[1] Melting points are corrected.
[2] Prepared by Dr. J. F. Mead.

scribed for the preparation of ethyl 2-phenylcinchoninate. Instead of distilling the residue left by removal of the ether, recrystallization was effected from 125 ml. of absolute ethanol, giving large, brownish yellow prisms, m.p. 105-107° at 1°/min. from 70°.

(6-Methoxy-2-phenylquinolyl-4) α-bromo-ε-aminoamyl ketone dihydrobromide. Sodamide was prepared from 26 g. (1.13 mole) of sodium according to the directions of Sargent and Buchman.

To the powdered sodamide in a 3-necked, 3 l. flask equipped with a Hirshberg stirrer and a condenser protected with a soda-lime tube, a solution of 276 g. (0.90 mole) of ethyl 2-phenylquininate[3] and 237 g. (0.90 mole) of ethyl ε-benzamidocaproate in 500 ml. of dry benzene was added. With continuous stirring the mixture was heated in a bath at 90° for 25 hrs. The bath was then removed.

The reaction mixture was cooled in an ice water bath while a solution of 700 ml. of conc. hydrochloric acid and 500 ml. of water was added. The stirrer was replaced with a stillhead, and the benzene was steam distilled until the temperature of the vapors reached 108°. After substituting a condenser for the still head, refluxing was continued for 40 hrs. The solution was then cooled, diluted with an equal volume of water, and made alkaline (pH 7.5) with 50 wt.% sodium hydroxide. The ketone was extracted with 2000

[3] Obtained from Dr. R. C. Elderfield of Columbia University.

ml. of chloroform and the solution was suction filtered to remove insoluble phenylquininic acid. After drying over sodium sulfate, the chloroform solution was concentrated under reduced pressure to 750 ml. and extracted with 760 g. of 40% hydrobromic acid. Chloroform was removed from the aqueous phase by warming and stirring, and the increase in weight was then found to be 160 g.

To this solution of the ketone 64.5 g. of bromine (88% of the theory required by the increase in weight) in 130 ml. of 40% hydrobromic acid were added rapidly at 80-85° with mechanical stirring. The reaction mixture was stirred 10 min., heated to the boiling point, rapidly filtered through a sintered glass funnel to remove some free acid (25 g.), and chilled in the cold room overnight. The product was collected on a sintered glass funnel, washed with i-propanol, acetone, and ether, and then dried to give 101 g. of a light yellow powder, m.p. 170-171° (decomp.) at 1°/min. from 154°. By concentration of the mother liquors a second crop of 27 g. decomposing 8° lower was obtained. The total yield was 128 g. (23%).

A sample recrystallized twice from methanol-i-propyl ether was obtained as light yellow clusters of needles, m.p. 174-175° (decomp.) at 1°/min. from 160°.

$C_{22}H_{23}O_2N_2Br.2HBr.2H_2O$	Calc.	C	42.3	H	4.7	N	4.5
(625.2)	Found	C	42.5	H	4.7	N	4.4

By acidifying the aqueous phase from the chloroform extraction of the ketone, washing the precipitate with ethanol to remove benzoic acid, and combining the residue with the other fractions which had been filtered off, 128 g. of crude 2-phenylquininic acid were recovered.

(6-Methoxy-2-phenylquinolyl-4)-α-piperidylcarbinol dihydrochloride. To a suspension of 104 g. (0.167 mole) of bromoketone salt in 1750 ml. of absolute ethanol in a 4 l. bottle, 490 ml. of 15 wt.% sodium carbonate were added. After displacing the air with nitrogen, the bottle was stoppered and shaken mechanically for 50 min. 2.5 g. of platinum oxide were then added, and the bottle was filled with hydrogen. Reduction, at room temperature under atmospheric pressure of hydrogen, was allowed to proceed for 3 hrs., at which time the rate of hydrogen absorption had fallen from 120 ml./min. initially to 0.5 ml./min. The total uptake was 4.45 liters; theory required 4.85 liters.

The catalyst and precipitated salts were filtered off, and the ethanol was removed under reduced pressure. A solid separated which was washed with water and dissolved in ethanol. The addition of excess ethanolic hydrochloric acid precipitated the yellow dihydrochloride which was washed with ethanol and acetone and dried to give 48.8 g., m.p. 240° (decomp.) at 0.6°/min. from 225°.

Purification could be effected only over the free base

which was obtained by neutralizing an alcoholic suspension of the dihydrochloride with 5 N sodium hydroxide, removing the solvent, and recrystallizing the residue from methanol. The crystals were stout rods or flat prisms containing one molecule of solvent and softening from 88-95°, partially fusing from 95-105°, then resolidifying and melting at 176.5-177.5° at 0.5°/min.

$C_{22}H_{24}O_2N_2 \cdot CH_3OH$	Calc.	C	72.6	H	7.4	N 7.4
(380.5)	Found	C	72.8	H	7.7	N 7.6

From i-propanol the free base crystallized unsolvated as clusters of colorless, feathery needles, m.p. 175.5-176.5° at 1°/min. from 160°.

$C_{22}H_{24}O_2N_2$	Calc.	C	75.8	H	6.9	N 8.0
(348.4)	Found	C	75.5	H	7.2	N 8.2

The monohydrobromide crystallized from 96% ethanol as colorless clusters of needles, m.p. 213.5-215° (decomp.) at 0.6°/min. from 195°.

$C_{22}H_{24}O_2N_2 \cdot HBr$	Calc.	C	61.5	H	5.9	N 6.5
(429.4)	Found	C	61.5	H	6.4	N 6.4

The dihydrochloride, precipitated from ethanol as small clusters of yellow needles, melted at 240.5-241° (decomp.) at 0.6°/min. from 225°, and analyzed for a hemihydrate[4].

$C_{22}H_{24}O_2N_2 \cdot 2HCl \cdot \frac{1}{2}H_2O$	Calc.	C	61.4	H	6.3	N 6.5
(430.4)	Found	C	61.4	H	6.4	N 6.8

[4] The analytical sample was prepared by Dr. J. F. Mead.

(8-Methoxy-2-Phenylquinolyl-4)-α-Piperidylcarbinol III

and

(8-Hydroxy-2-Phenylquinolyl-4)-α-Piperidylcarbinol IV

8-Methoxy-2-phenylcinchoninic acid was prepared from o-anisidine, pyruvic acid, and benzaldehyde according to the directions of Doebner (7), a slightly improved yield (27%) being obtained by the addition of a small quantity of conc. sulfuric acid (10). The acid was esterified in 81 per cent yield with ethanol and sulfuric acid.

Ethyl 8-methoxy-2-phenylcinchoninate was condensed with ethyl ε-benzamidocaproate in benzene using sodamide as the condensing agent, and the product was hydrolyzed with 6 N hydrochloric acid to give 44 per cent of crude (8-methoxy-2-phenylquinolyl-4) ε-aminoamyl ketone isolated as the dihydrobromide. The reaction of this product with bromine in hydrobromic acid gave the bromoketone in 70 per cent yield.

The piperidine ring was then closed in aqueous ethanol with sodium carbonate, and the resulting piperidyl ketone was reduced immediately with platinum oxide and hydrogen to give an 80 per cent yield of the methoxyquinolylpiperidylcarbinol isolated as the free base.

The 8-methoxy group was hydrolyzed by refluxing with 48 per cent hydrobromic acid for 24 hours. The hydroxyquin-

olypiperidylcarbinol was isolated as the sodium salt in 90 per cent yield, from which it was converted to the free base with an equivalent of hydrochloric acid.

Experimental Part[1]

8-Methoxy-2-phenylcinchoninic acid (7). A solution of 540 g. (3.07 moles) of 50% aqueous pyruvic acid, 320 g. (3.02 moles) of benzaldehyde, and 370 g. (3.01 moles) of o-anisidine in 5.0 l. of 96% ethanol containing 3 ml. of conc. sulfuric acid was refluxed for 5 hrs. 4.0 l. of ethanol was distilled off at atmospheric pressure (3½ hrs.) and the product was allowed to crystallize. Filtering, washing with ethanol, and drying in the oven at 100° gave 204 g. of yellow prisms, m.p. 211-213° at 1°/min. By concentrating the mother liquors to ½ volume (1000 ml.) and recrystallizing the deposited crystals from ethanol, a second crop of 24 g., m.p. 209-212°, was obtained to give a total yield of 228 g. (27%).

Ethyl 8-methoxy-2-phenylcinchoninate. To a suspension of 226 g. (0.81 mole) of 8-methoxycinchophen in 1150 ml. of absolute ethanol, 115 ml. of conc. sulfuric acid were added. The mixture was refluxed for 12 hrs., a clear solution being obtained in 10 min. Most of the ethanol (900 ml.) was then removed at ordinary pressure over a 12 hr. period. The residual syrup was poured over 2 l. of ice and water, and the reaction mixture was made basic (pH 9) in the cold with 50 wt.%

[1] Melting points are corrected.

sodium hydroxide. The ester was then extracted with 700, 300, and 200 ml. portions of chloroform. The chloroform solution was dried over sodium sulfate and then evaporated to dryness under reduced pressure. The crystalline residue was recrystallized from 600 ml. of absolute ethanol, filtered, washed with ethanol, and dried in the vacuum oven at 70° and then over phosphorus pentoxide to give 201 g. (81%) of large, pale yellow prisms, m.p. 106-108° at 1°/min. from 95°. An analytical sample recrystallized from ethanol melted at 106.5-107.5°.

$C_{19}H_{17}O_3N$	Calc.	C	74.3	H	5.6	N 4.6
(307.3)	Found	C	74.6	H	5.6	N 4.8

By acidification of the aqueous phase from the chloroform extraction, 26 g. of free acid were recovered.

(8-Methoxy-2-phenylquinolyl-4) ∈-aminoamyl ketone dihydrobromide. Sodamide was prepared from 33.5 g. (1.46 mole) of sodium according to the directions of Sargent and Buchman (5).

To the powdered sodamide in a 3-necked, 5 l. flask equipped with a Hirshberg stirrer and a condenser protected with a soda-lime tube, a warm (70°) solution of 291 g. (0.95 mole) of ethyl 8-methoxy-2-phenylcinchoninate and 252 g. (0.95 mole) of ethyl ∈-benzamidocaproate in 560 ml. of dry benzene was added. Stirring was begun with a powerful motor, and the flask was heated in an oil bath at 100-110° for 23

hrs. The mixture became very thick after 30 min.; stirring was stopped and then started again after 8 hrs.

The reaction mixture was cooled in an ice water bath while a solution of 725 ml. of conc. hydrochloric acid and 500 ml. of water was added. The stirrer was then replaced with a stillhead, and the benzene was steam distilled until the temperature of the vapors reached 108°. After substituting a condenser for the stillhead and replacing the 225 ml. of aqueous distillate with an equal volume of 6 N hydrochloric acid, refluxing was continued for 29 hrs. The solution was then cooled, diluted with 500 ml. of water, made alkaline (pH 9) with 50 wt.% sodium hydroxide; and the ketone was extracted with 1000, 250, and 100 ml. portions of chloroform. The chloroform extract was washed twice with equal volumes of dilute sodium hydroxide solution to remove 8-methoxycinchophen, small quantities of 50% sodium hydroxide being added to the aqueous phase until it retained a permanent deep yellow color after shaking with the chloroform. After drying over sodium sulfate, the chloroform layer was evaporated to dryness under reduced pressure to give 235 g. of a dark brown oil. Upon addition of 150 ml. of 48% hydrobromic acid to the oil, a large quantity of chloroform boiled off. 500 ml. of acetone were added, and the solution obtained by warming was evaporated almost to dryness under reduced pressure. Upon

standing, the residual oil slowly crystallized. The solid was collected on sintered glass, washed with acetone, i-propanol, and acetone again, and dried over calcium chloride and sodium hydroxide *in vacuo* to give 177 g. of a deep yellow powder, m.p. 102-108° at 1.5°/min. from 85° with sintering from 95-101°. From the mother liquors and washings, by similar treatment, a second crop of 42 g., m.p. 102-106° under the above conditions, was obtained to give a total yield of 219 g. (44%).

A sample of 0.101 g. of this ketone salt required 19.40 ml. of 0.0200 N silver nitrate to completely precipitate the bromine. The product was therefore the *di*-hydrobromide, 0.101 g. of which would require 19.60 ml. of silver nitrate.

Recrystallization from 10% hydrobromic acid by addition of acetone gave yellow clusters of prisms, m.p. 103-111°. The compound was not submitted for analysis.

By acidifying the aqueous phase from the chloroform extraction and recrystallizing the precipitate from 1000 ml. of ethanol, 84 g. of 8-methoxycinchophen were recovered.

(8-Methoxy-2-phenylquinolyl-4) α-bromo-ε-aminoamyl ketone dihydrobromide. To a solution of 219 g. (0.43 mole) of the ketone salt in 200 ml. of 48% hydrobromic acid at 100°, 63.5 g. (0.40 mole) of bromine in 100 ml. of 48% hydrobromic acid were added dropwise with mechanical stirring over a 15 min.

period. The reaction mixture was then heated to boiling and allowed to cool overnight. The large clusters of yellow prisms which separated were collected on a sintered glass funnel, washed once with water, then abundantly with acetone, and finally with ether. After drying over calcium chloride and sodium hydroxide at 30 mm., 194 g. (70%) of a deep yellow powder were obtained, m.p. 141.5-142° (decomp.) at 0.9°/min. from 130°. After three recrystallizations from 10% hydrobromic acid, a sample was obtained as clusters of long, yellow prisms, m.p. 146.5-147° (decomp.) at 0.6°/m. from 130°. The analysis showed it to be a tetrahydrate.

$C_{22}H_{23}O_2N_2Br.2HBr.4H_2O$	Calc.	C	40.0	H	5.0	
(661.3)	Found	C	40.2	H	5.1	

The loss of water by further drying caused the compound to become orange in color and the decomposition point to be depressed 2°. The analysis then showed that only two molecules of water of crystallization remained.

$C_{22}H_{23}O_2N_2Br.2HBr.2H_2O$	Calc.	C	42.3	H	4.7	Ionic Br	38.3
(625.2)	Found	C	41.9	H	5.3	Ionic Br	38.1

<u>(8-Methoxy-2-phenylquinolyl-4)-α-piperidylcarbinol</u>. To a solution of 110 g. (0.167 mole) of bromoketone salt in 1800 ml. of absolute ethanol, 490 ml. of 15 wt.% sodium carbonate were added. After displacing the air with nitrogen, the bottle was stoppered and shaken mechanically for 1 hr.

1.0 g. of platinum oxide was then added, and the bottle was filled with hydrogen. The reduction at room temperature under atmospheric pressure of hydrogen, was allowed to proceed for 4½ hrs., at which time the rate of hydrogen absorption had fallen from 360 ml./min. initially to 0.8 ml./min. The total uptake was 5.3 l.; theory required 4.6

The catalyst and precipitated salts were filtered off, and the ethanol was removed under reduced pressure. The solid which separated was filtered and washed with ethanol, water, ethanol, and ether. After drying, it weighed 40.8 g., m.p. 195-198° at 1.5°/min. from 180°. A second crop of 6.2 g. was obtained from the mother liquors to give a total yield of 46.5 g. (80%). Recrystallization from 96% ethanol gave colorless needles, m.p. 196-200° at 1°/min. from 180°. Further recrystallizations from several different solvents gave crystals of variable melting point in the range of 193-205°, depending upon the particular sample of compound at hand. Analytical samples were obtained melting at 194-196° and 197-202° at 1°/min.

$C_{22}H_{24}O_2N_2$	Calc.	C	75.8	H	6.9	N	8.0
(348.4)	Found	C	75.9	H	7.1	N	8.0 (194-6°)
			76.1		7.2		8.2 (197-202°)

The monohydrobromide crystallized from 96% ethanol as colorless clusters of needles, m.p. 215-217° (decomp.) at 1°/min. from 190°.

$C_{22}H_{24}O_2N_2.HBr$	Calc.	C	61.5	H	5.9	N 6.5
(429.3)	Found	C	61.5	H	6.2	N 6.8

(8-Hydroxy-2-phenylquinolyl-4)-α-piperidylcarbinol. 23.5 g. (0.068 mole) of the 8-methoxycarbinol were refluxed with 120 ml. of 48% hydrobromic acid for 24 hrs. The reaction mixture was cooled, and the crystals which separated were collected, washed with water and acetone, and dried to give 27.0 g. of the dihydrobromide salt of the 8-hydroxycarbinol. This material was suspended in 1350 ml. of hot 96% ethanol, 10 ml. of 50 wt.% sodium hydroxide were added, and the resulting solution was filtered and concentrated at atmospheric pressure to 700 ml. On chilling overnight, rosettes of yellow needles of the sodium salt separated which were filtered off, washed with ethanol and ether, and dried in the vacuum oven at 70° to give 17.6 g., m.p. above 320° (decomp.). Concentration of the mother liquors gave an additional 4.6 g. of product, the total yield being 22.2 g. (ca.90%). Analysis gave inconsistent results.

17.6 g. of the sodium salt were suspended in 400 ml. of 96% ethanol, and, at the boiling point, 53 ml. of 0.92 N hydrochloric acid were added. On boiling for 1 min., crystals began to separate from the clear solution. After chilling overnight, the pale yellow needles were collected, washed with ethanol, and dried to give 11.4 g., m.p. 200-

202° at 1°/min. from 180°. After recrystallization from ethanol, a sample melted at 201-202°. A dark green color reaction was obtained with ferric chloride.

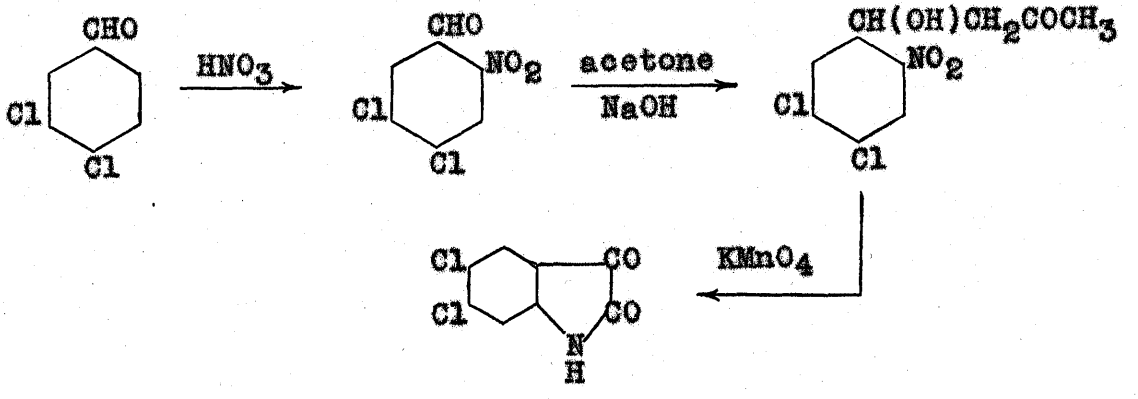
$C_{21}H_{22}O_2N_2$	Calc.	C	75.4	H	6.6	N 8.4
(334.4)	Found	C	75.5	H	6.8	N 8.3

The monohydrochloride crystallized from 38% ethanol as clusters of long prisms, m.p. 227.5-228° (decomp.) at 1.5°/min. from 220°.

$C_{21}H_{22}O_2N_2.HCl$	Calc.	C	68.0	H	6.3	Ionic Cl 9.6
(370.9)	Found	C	68.0	H	6.1	Ionic Cl 9.8

(6,7-Dichloro-2-Phenylquinolyl-4)-α-Piperidylcarbinol V

6,7-Dichloro-2-phenylcinchoninic acid could not be prepared by the Doebner reaction (11), and attention was therefore directed to the synthesis of 5,6-dichloroisatin. The preparation of this compound by the following series of reactions was reported (12):



The yields were not given, and investigation of the synthesis showed that step two, the condensation of the aldehyde with acetone, gave yields of less than 10 per cent. This method was therefore not practicable for large scale preparations. The synthesis of 5,6-dichloroisatin was also reported by Sandmeyer (13), who obtained it as one of the products in the ring closure of 3,4-dichloroisonitrosoacetanilide. However, no yield was reported here either, and it was found upon investigation (11) that the predominant product of this reaction was 4,5-dichloroisatin and that the isomers were exceedingly difficult to separate.

Since chlorination and bromination of isatin in aqueous solution give almost quantitative yields of the 5-haloisatin, and since a simple method of preparing 6-chloroisatin from m-chloroaniline was available (14), it was considered likely that the required substituted isatin could be made by direct chlorination of 6-chloroisatin. This method proved to be successful and gave crude 5,6-dichloroisatin in 50 to 60 per cent yield. The structure was confirmed by oxidation of a sample with alkaline hydrogen peroxide to 4,5-dichloroanthranilic acid according to the method described by Sumpter and Jones (15).

The chlorination reaction appeared to be surface catalyzed. Runs of more than 40 g. gave appreciably decreased

yields, e.g. with 130 g. the yield was 29 per cent; with 60 g., 43 per cent. The best yield (60%) was obtained by the addition of powdered glass to the reaction mixture. The reaction was also exothermic, and slight improvements were effected by keeping the temperature below 20°.

6,7-Dichloro-2-phenylcinchoninic acid was then prepared by employing the Pfitzinger condensation with acetophenone. The usual 100 per cent excess of the latter reactant led to the formation of a considerable quantity of a deep red-colored by-product. Reducing this excess to only 20 per cent gave much improved yields (86%), and it is probable that still further reduction might effect even greater improvement.

The red-colored compound analyzed very closely for $C_{24}H_{17}O_3NCl_2$, indicating a condensation of two molecules of acetophenone and one of the dichloroisatin with the removal of one molecule of water. The substance was alkali soluble, but its insolubility in both aqueous bicarbonate and ammonia permitted it to be easily separated from the dichlorocinchophen.

The 6,7-dichlorocinchophen was esterified with ethanol and sulfuric acid in 80 per cent yield.

Ethyl 6,7-dichloro-2-phenylcinchoninate was condensed with ethyl ϵ-benzamidocaproate in benzene using sodamide

as the condensing agent, and the product was subjected to hydrolysis with 30 vol. per cent sulfuric acid. In working up the reaction mixture, there was encountered a large amount of by-product which prevented the ketone from being extracted with chloroform. It was found then that a fairly effective separation could be made immediately after hydrolysis by exhaustive extraction of the residue with boiling 10 vol. per cent sulfuric acid. Isolation of the aminoketone from the combined extractions as the crystalline monohydrobromide gave a yield of 30 per cent.

The reaction of this product with bromine in hydrobromic acid gave the bromoketone in 90 per cent yield. The extreme insolubility of the product made this step difficult to perform. The bromoketone salt, until thoroughly dry, was found to separate as a gel from all solvents except 48 per cent hydrobromic acid; it was also soluble in acetone as long as any excess hydrobromic acid was present. For these reasons, the isolation and purification of this product required considerable care.

A note may be made here concerning the by-product mentioned above. Analysis did not suggest any reasonable structure, but from the fact that experiments designed to isolate the benzoic acid formed during hydrolysis never gave more than 55 per cent of the theoretical quantity,

it is believed that this by-product involved condensation of the benzamido group to form a product not hydrolyzed under the conditions employed.

Piperidine ring closure and reduction to the carbinol in the usual manner gave very poor yields (12%), an appreciable quantity of by-product, which is still uncharacterized, precipitating during the hydrogenation. Isolation of the piperidyl ketone prior to reduction resulted in only a slight improvement, and only when small (5 g.) quantities of bromoketone were employed were greater yields (25%) obtained.

Experimental Part[1]

5,6-Dichloroisatin (12, 13). A suspension of 30.0 g. (0.165 mole) of powdered (60 mesh) 6-chloroisatin[2] in 400 ml. of glacial acetic acid in a round bottom, 3-necked, 1 liter flask fitted with a mechanical glass stirrer, a gas inlet tube that dipped below the surface of the liquid, and an outlet tube, was cooled in a water bath at 18-20°. Then, with vigorous stirring, chlorine was passed in for 3 hrs. while keeping the bath at the above temperature. The chlorination was stopped, and air was passed through the reaction mixture for 30 min. to remove some of the excess chlorine. 200 ml. of glacial acetic acid were then added, and

[1] Melting points are corrected.
[2] Prepared by A. E. Senear.

the solid was dissolved by refluxing. On cooling, the product crystallized in large orange red prisms. After filtering, washing with acetic acid and ligroin (30-60), and drying in the vacuum oven at 70°, 19.2 g. (54%) were obtained, m.p. 266-272° (decomp.) at 1°/min. from 250°. After another crystallization from acetic acid, the melting point was 274-277° under the above conditions. The literature (12) gives the melting point as 273-275°.

To 0.150 g. of the above compound, 2.8 ml. of 10% sodium hydroxide were added; the suspension of yellow solid obtained by warming was oxidized by slowly adding 2.8 ml. of 3% hydrogen peroxide solution. The clear, pale yellow solution was allowed to stand for 1 hr., and the acid was then precipitated with 6 N hydrochloric acid, washed three times with water, and recrystallized twice from dilute acetic acid to give colorless needles, m.p. 213.5-214.5°. The literature (16) gives the melting point of 4,5-dichloroanthranilic acid as 213-214°, whereas the 3,4-isomer melts at 237-238°.

6,7-Dichloro-2-phenylcinchoninic acid. To a suspension of 75.1 g. (0.35 mole) of 5,6-dichloroisatin in 415 ml. of absolute ethanol, 50 g. (0.42 mole) of acetophenone were added followed by 208 ml. of 33 wt.% potassium hydroxide. The deep purple reaction mixture was refluxed

for 8 hrs. in a water bath at 95°. The solvents were then removed by evaporating overnight in a large dish on a hot water bath. The solid cake was almost completely dissolved in 2 l. of hot water, and, after cooling to 40°, the mixture was extracted with 1250 ml. of i-propyl ether in two portions. After removal of the dissolved ether by warming in a water bath, the solution was strongly acidified with conc. hydrochloric acid[3]. The deep orange-colored precipitate was filtered, washed with water, suspended in 2 l. of water, and dissolved by adding conc. ammonia solution. The solution was stirred until almost clear and then suction filtered from an insoluble, deep red by-product. The filtrate was acidified with conc. hydrochloric acid[3], and the product was then collected and washed by suspension in one liter of water. Drying on clay and then at 100° under reduced pressure gave 95 g. (86%), m.p. 275-276° (decomp.) at 1.5°/min. from 240°. For analysis, a sample was recrystallized from ethanol to give almost colorless clusters of prisms, m.p. 277-278° (decomp.) at 0.9°/min. from 250°.

$C_{16}H_9O_2NCl_2$	Calc.	C	60.4	H	2.9	N 4.4
(318.2)	Found	C	60.1	H	3.0	N 4.5

The by-product, after three recrystallizations from ethanol, was obtained as flat, red prisms, m.p. 238°

[3] It was subsequently found that when the acid was precipitated from the hot aqueous solution, the precipitate was much less gelatinous in nature.

(decomp.) at 0.5°/min. from 220°.

$C_{24}H_{17}O_3NCl_2$	requires	C	65.7	H	3.7	N 3.4
	Found	C	65.8	H	3.9	N 3.2

Ethyl 6,7-dichloro-2-phenylcinchoninate. To a suspension of 54 g. (0.17 mole) of 6,7-dichlorocinchophen in 270 ml. of absolute ethanol, 27 ml. of conc. sulfuric acid were added. The mixture was refluxed for 21 hrs., a clear solution being obtained in 90 min. After evaporating the solution to 1/3 volume at ordinary pressure, 500 ml. of ice water were added, and the mixture was made basic (pH 9) in the cold with 15 wt.% potassium hydroxide. The ester was then extracted with 600 and 150 ml. portions of chloroform. The chloroform solution was dried over sodium sulfate and then evaporated to dryness under reduced pressure. The crystalline residue was recrystallized from 1500 ml. of 96% ethanol, filtered, washed with ethanol and ligroin (30-60), and dried in the vacuum oven at 70° to give 38.9 g. of colorless needles, m.p. 115-118° at 0.8°/min. from 100°. A second crop of 7.4 g., m.p. 113-117°, was obtained by concentration of the mother liquors, giving a total yield of 46.3 g. (79%). The melting point remained unchanged through four recrystallizations from ethanol. An analytical sample, prepared by recrystallization from ligroin (86-100) and then ethanol, melted at 115° at 1°/min. from 110°.

$C_{18}H_{13}O_2NCl_2$	Calc.	C	62.4	H	3.8	N 4.1
(346.2)	Found	C	62.2	H	4.1	N 4.1

(6,7-Dichloro-2-phenylquinolyl-4) ϵ-aminoamyl ketone hydrobromide. Sodamide was prepared from 9.2 g. (0.40 mole) of sodium according to the directions of Sargent and Buchman (5).

To the powdered sodamide in a 3-necked, 500 ml. flask equipped with a Hirshberg stirrer and a condenser protected with a soda-lime tube, a warm (45°) solution of 70.1 g. (0.20 mole) of ethyl 6,7-dichloro-2-phenylcinchoninate and 57.3 g. (0.22 mole) of ethyl ϵ-benzamidocaproate in 125 ml. of dry benzene was added. With continuous stirring the mixture was heated in a bath at 100-110° for 22 hrs.

To the cold reaction mixture, a warm (50°) solution of 150 ml. of conc. sulfuric acid and 300 ml. of water was added. The stirrer was replaced with a stillhead, and the benzene was steam distilled. After substituting a condenser for the stillhead and replacing the 40 ml. of aqueous distillate with an equal volume of water, refluxing was continued for 89 hrs. The reaction mixture was then diluted with 1250 ml. of water and heated to the boiling point, giving an insoluble dark oil and a yellow brown solution. The oil was removed by suction filtering through a steam-jacketed funnel, and the filtrate was chilled and made alkaline with 50 wt.% sodium hydroxide keeping the temperature below 40° by cooling in an ice bath.

The ketone was extracted with 500, 200, and 100 ml. portions of chloroform; and the organic layer was then washed with 500 ml. of water, dried over sodium sulfate, and evaporated to dryness under reduced pressure to give 15.9 g. of a dark brown oil. To this oil 15 ml. of 40 wt.% hydrobromic acid and 50 ml. of acetone were added. A clear solution was obtained by gently warming for 15 min., and the solvents were then completely removed under reduced pressure. The residue was crystallized from 50 ml. of 96% ethanol. Filtering, washing with ethanol, then acetone and ether, and drying in the vacuum oven at 70° gave 10.9 g. of yellow needles.

The residue from the sulfuric acid extraction, which hardened on cooling, was reextracted with eight 500 ml. portions of 10 vol.% sulfuric acid and worked up in the manner described to give an additional 20.2 g. of yellow needles, the total yield being 31.1 g.

The melting points of the various batches varied from 162-165° for the first crop to 168-174° for later crops. This material was brominated without further purification.

A sample of the compound, recrystallized from 96% ethanol to constant melting point, was obtained as faintly colored needles, m.p. 168-181° at 1°/min. from 150°. Recrystallization from acetonitrile or acetic acid did not improve the melting point. The analysis was approximately correct

for a monohydrobromide monohydrate.

$C_{21}H_{20}ON_2Cl_2.HBr.H_2O$	Calc.	C	51.9	H	4.8
(486.2)	Found	C	52.4	H	5.1

(6,7-Dichloro-2-phenylquinolyl-4) ∝-bromo-∈-aminoamyl ketone dihydrobromide. To 130 ml. of 48% hydrobromic acid, 18.0 g. (0.037 mole) of ketone salt were added. The mixture was heated to the boiling point and a solution of 5.9 g. (0.037 mole) of bromine in 12 ml. of 48% hydrobromic acid was added dropwise over a 20 min. period with constant stirring. The mixture became so thick due to crystallization of the product that 45 ml. of 48% hydrobromic acid were added during the addition. When all the bromine had been added, the reaction mixture was heated at the boiling point for 10 min. After chilling overnight, the product was filtered, pressed as dry as possible, and then washed abundantly with acetone. After drying in vacuo over phosphorus pentoxide, 21.4 g. (90%) of yellow prisms were obtained, m.p. 227-228° (decomp.) at 1°/min. from 170°. Recrystallization of a sample from glacial acetic acid did not alter the melting point.

$C_{21}H_{19}ON_2Cl_2Br.2HBr$	Calc.	C	40.2	H	3.4
(628.1)	Found	C	40.1	H	3.5

(6,7-Dichloro-2-phenylquinolyl-4)-∝-piperidylcarbinol. To a suspension of 18.5 g. (0.029 mole) of bromoketone salt in

370 ml. of water and 740 ml. of ether in a 2 l. bottle, 220 ml. of 15 wt.% sodium carbonate were added. After displacing the air with nitrogen, the bottle was stoppered and shaken mechanically for 50 min., giving an orange ether layer and a colorless aqueous layer. Considerable pressure had developed. The ether layer was dried over sodium sulfate and evaporated to dryness at reduced pressure under nitrogen, and the 10.5 g. of residue were taken up in 200 ml. of absolute ethanol and reduced in the usual manner with 0.7 g. of platinum oxide. After 2½ hrs. the hydrogen uptake was 765 ml., the rate having fallen to 0.5 ml./min. from the 70 ml./min. initially. Theory required 925 ml. The ethanol solution was filtered and concentrated under reduced pressure to 80 ml. 3 ml. of 6 N ethanolic hydrochloric acid were then added, and, after standing overnight, the light pink precipitate which separated was filtered, washed with ethanol, acetone, and ether, and dried to give 2.25 g. (17%) of the dihydrochloride, m.p. 227° (decomp.) at 1°/min. from 210°.

This material was suspended in 100 ml. of 96% ethanol, and, at the boiling point, 0.6 ml. of 50 wt.% sodium hydroxide was added. The solution was filtered with Norite and chilled overnight. The crystals which separated were collected, washed with ethanol, and dried to give 1.5 g. of

light buff-colored flat prisms, m.p. 202-206° at 1°/min. from 185° after effervescence and resolidification. A sample, recrystallized for analysis from 96% ethanol, was obtained as colorless prisms, m.p. 204-206° under the conditions recorded above.

$C_{21}H_{20}ON_2Cl_2 \cdot C_2H_5OH$	Calc.	C	63.7	H	6.1	N 6.5
(433.4)	Found	C	64.0	H	6.4	N 6.3

Drying at 100° <u>in vacuo</u> removed the ethanol of crystallization.

$C_{21}H_{20}ON_2Cl_2$	Calc.	C	65.1	H	5.2	N 7.2
(387.3)	Found	C	64.8	H	5.5	N 7.0

The monohydrochloride crystallized as pale greenish-yellow needles from an ethanolic solution to which one or two equivalents of ethanolic hydrochloric acid had been added, m.p. 235.5-237° (decomp.) at 0.7°/min. from 200°.

$C_{21}H_{20}ON_2Cl_2 \cdot HCl \cdot \frac{1}{2}H_2O$	Calc.	C	58.3	H	5.1	N 6.5
(432.8)	Found	C	58.7	H	5.1	N 6.1

The dihydrochloride was obtained as light yellow microcrystalline needles, m.p. 229-230° (decomp.) at 1°/min. from 205°, when a large excess of hydrochloric acid was added to an ethanolic solution of the base.

$C_{21}H_{20}ON_2Cl_2 \cdot 2HCl$	Calc.	C	54.8	H	4.8	Ionic Cl	15.4
(460.2)	Found	C	55.2	H	5.0	Ionic Cl	15.2

(+)-2'-Phenyl-3-Ethylruban-9-ol

Phenyllithium was added to dihydrocinchonine prepared from cinchonine in 93 per cent yield by catalytic reduction with palladium, according to the directions of Skita and Franck (17). Upon treating the addition product with water, the lithium rest was hydrolyzed and hydrogen was split out to give the required substituted ruban in 20 per cent yield.

The position of the phenyl substituent was confirmed by chromic acid oxidation of a sample, according to the method of John (18), to 2-phenylcinchoninic acid.

Experimental Part[1]

Dihydrocinchonine (17). From 30 g. (0.10 mole) of cinchonine, 27.8 g. (93%) of dihydrocinchonine were prepared following the directions of Skita and Franck. The compound was obtained as colorless needles, m.p. 270.5-273° (decomp.) at 4°/min. from 250°, after recrystallization from 650 ml. of benzene using a Soxhlet extractor.

(+)-2'-Phenyl-3-ethylruban-9-ol. A solution of 0.020 mole of phenyllithium in 30 ml. of ether, prepared according to the directions of Gilman, Zoellner, and Selby (19), was added to a suspension of 5.9 g. (0.020 mole) of dihydrocinchonine in 40 ml. of dry ether in an ice water bath at 0-5°. The reaction mixture was mechanically stirred. After 5 min., the

[1] Melting points are corrected.

bath was removed; stirring was contined for 30 min., during which time the mixture turned dark yellow-green and most of the solid dissolved. The reaction mixture was then poured into 500 ml. of ice water, and the ether was removed by warming on the steam bath. The insoluble solid was collected, washed with water, dried, and extracted with 500 ml. of ether. The ether was removed on the steam bath, and the residue was then recrystallized from 96% ethanol. Two crops, obtained by concentration of the solution and the mother liquors, weighed a total of 2.7 g., m.p. 254-257° (decomp.). Recrystallization from 96% ethanol gave colorless needles, m.p. 260-261° (decomp.) at 2°/min. from 245°. In subsequent runs, the product showed a much greater solubility in ethanol when crude than after initial purification, making it necessary to concentrate the first ethanol solution to a very small volume in order to obtain the product. The average yield from 6 runs was 20%.

$C_{25}H_{28}ON_2$	Calc.	C	80.6	H	7.6	N	7.5
(372.5)	Found	C	80.5	H	7.7	N	7.7

$$\text{Spec. Rot. } [\alpha]_D^{25^\circ} = \frac{+1.30^\circ \times 5.00}{1.00 \times 0.0505} = +129^\circ \text{ *}$$

* (1 mole of base + 4 moles of HCl in water, c = 1%)

To a solution of 1.00 g. of the above compound in 20 ml.

of water containing 1.88 ml. of conc. sulfuric acid and 0.2 g. of powdered manganese dioxide, a solution of 2.0 g. of chromic anhydride in 2 ml. of water was added dropwise, at the boiling point and with shaking, over a 25 min. period. The mixture was then boiled for 5 min., diluted with 120 ml. of boiling water, and made alkaline with conc. ammonia solution (10 ml.). After 1 hr. of digestion on the steam bath, it was filtered with Cellite; and the precipitate was washed well with 70 ml. of boiling water containing 10 ml. of conc. ammonia. The filtrate and washings were concentrated to 30 ml., filtered, and acidified (pH 3) with hydrochloric and acetic acids. The precipitate which separated on chilling overnight was collected, washed, and dried to give 0.310 g. (46%) of colorless needles, m.p. 212-216° at 1°/min. from 195°. After recrystallization from ethanol, the melting point was 214-215°. The mixed melting point with a sample of 2-phenylcinchoninic acid (m.p. 214-216°) was 214-216°.

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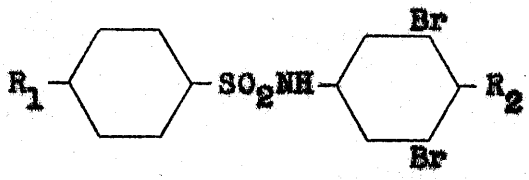
Sulfonamides

Great interest was shown in sulfonamides as antimalarials following the observation by Coggeshall (1a) that a single oral dose of sulfanilamide completely and permanently cured the highly fatal P. knowlesi infection in rhesus monkeys. Other sulfonamides, such as sulfadiazine, have definite therapeutic action in human malarias (1b,c,d). For a period of time, it was thought that this type of drug might be the answer to the long search for a true causal prophylactic, but further investigations revealed that these compounds did not have such an action. This disappointing result, coupled with other objectionable features of the sulfonamides, led to their being discarded in favor of more promising types of compounds.

The bacteriostatic action of the sulfanilamides is reversed by p-aminobenzoic acid. It is interesting to note that this action of the 3',5'-dibromo derivatives, which is two to four times as great as sulfadiazine against pneumococcus and Friedlander's bacillus in vitro, is not inhibited by p-aminobenzoic acid. This result indicates that they are involved in a mechanism which differs from that of the usual sulfa type drugs.

The three compounds prepared in this series and the steps in their synthesis are outlined below.



I 4-Amino-3',5'-dibromobenzenesulfonanilide

R_1 $-NH_2$

R_2 $-H$

II 4-Amino-4'-dimethylamino-3',5'-dibromobenzene-sulfonanilide

R_1 $-NH_2$

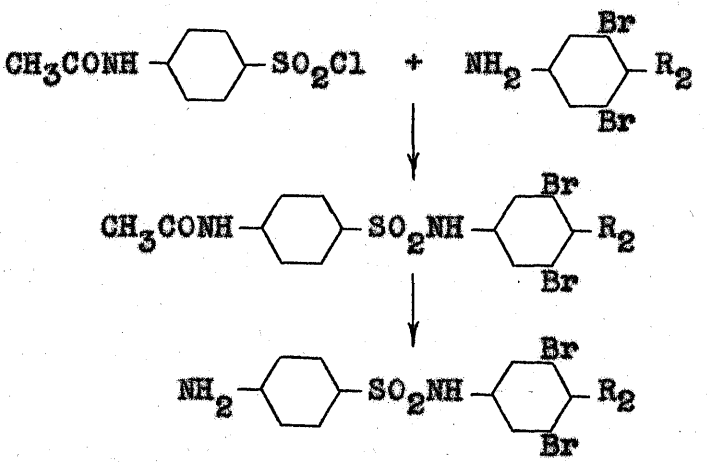
R_2 $-N(CH_3)_2$

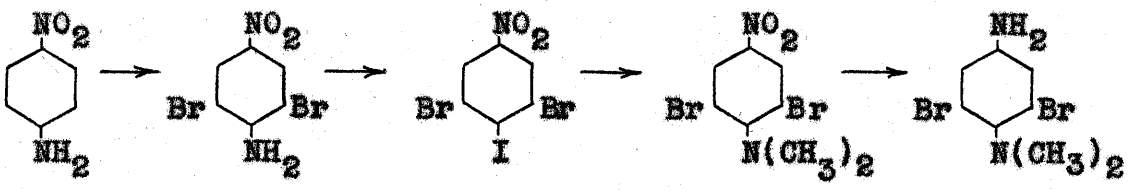
III 4-Aminomethyl-3',5'-dibromobenzenesulfonanilide

R_1 $-CH_2NH_2$

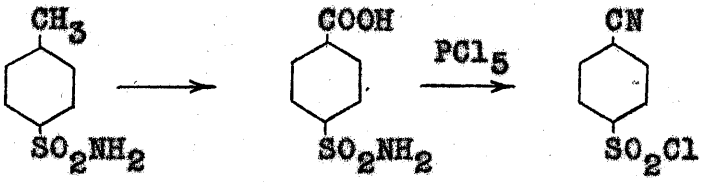
R_2 $-H$

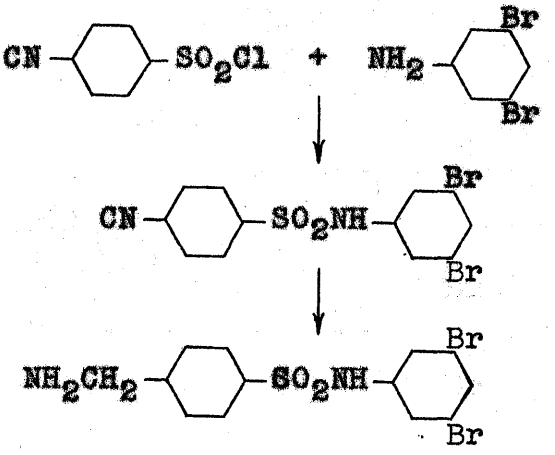
I and II





III





4-Amino-3',5'-dibromobenzenesulfonanilide (I)

This compound has already been prepared (2), but since no details were given, it seems advisable to describe the method of preparation employed here.

Acetylsulfanilyl chloride was coupled with 3,5-dibromaniline in pyridine solution following the method used by Long and Burger (3) to prepare a similar compound. The acetyl group was then removed by refluxing with an ethanol-hydrochloric acid mixture according to the procedure of the above authors (3).

The final compound was found to melt 5° higher than previously reported (2).

Subsequent to the completion of this work, the preparation of this compound was again reported (4). The melting point recorded this time was identical with that found here.

Experimental Part[1]

4-Acetylamino-3',5'-dibromobenzenesulfonanilide (2). To a solution of 36.1 g. (0.144 mole) of 3,5-dibromoaniline[2] in 110 ml. of dry pyridine, 50.6 g. (0.216 mole) of acetylsulfanilyl chloride were added in 10 portions over a 15 min. period. After standing at room temperature for one hour, the reaction mixture was heated on a water bath for 17

[1] Melting points are corrected.
[2] Prepared by Dr. J. F. Mead

hours, and then added dropwise, with stirring, to a mixture of 400 g. of ice, 1000 ml. of water, and 180 ml. of conc. hydrochloric acid. After chilling for one hour, the solid was filtered, washed with water, and dried in the oven at 90°. It was then dissolved in 2 N sodium carbonate solution (350 ml.), filtered, and precipitated with 4 N hydrochloric acid (250 ml.). After washing with water and drying, 60.2 g. (93%) of product, m.p. 242-244° was obtained. The reported melting point is 244°.

4-Amino-3',5'-dibromobenzenesulfonanilide (2). A solution of 60.2 g. (0.134 mole) of the acetyl compound, 1000 ml. of 96% ethanol and 300 ml. of conc. hydrochloric acid was refluxed for 1½ hrs. When cool, the solution was poured into 1600 ml. of water. The solution was brought to about pH 8 with conc. ammonia solution, and crystallization occurred. After filtering, washing with water, and drying, 43 g. (79%) of product, m.p. 149.5-152° was obtained. Recrystallization from 96% ethanol gave almost colorless, feathery clusters of needles melting at 154-155°.

$C_{12}H_{10}O_2N_2Br_2S$ (406.1)	Calc.	C	35.5	H	2.5	N 6.9
	Found	C	35.3	H	2.4	N 6.9

4-Amino-4'-dimethylamino-3',5'-dibromobenzenesulfonanilide (II)

The 4-dimethylamino-3,5-dibromonitrobenzene required for the synthesis of this compound could not be prepared by several commonly employed procedures (5,6). The bromination of p-nitro-N,N-dimethylaniline caused one methyl group to be removed and gave 4-nitro-2,6-dibromo-N-methylaniline (7). Attempted methylation of 4-nitro-2,6-dibromaniline with both methyl sulfate and formaldehyde were also unsuccessful (7,8).

The reaction of p-chloronitrobenzene with methylamine in a sealed tube to give p-nitro-N-methylaniline has been reported by Blanksma (9) and was successfully carried out in this laboratory with dimethylamine to give p-nitro-N,N-dimethylaniline (7). Accordingly, the reaction of 4-iodo-3,5-dibromonitrobenzene with dimethylamine was tried in a sealed tube at 120-130°. This method was entirely successful and gave the required compound in 85 per cent yield.

The catalytic reduction of 4-dimethylamino-3,5-dibromonitrobenzene using Raney nickel at 60° under 3 atm. pressure of hydrogen according to the method used by Drake (10) to reduce similar halogenated nitrobenzenes gave quantitative yields of 4-dimethylamino-3,5-dibromaniline. The free amine appeared to be unstable and was therefore immediately coupled with acetylsulfanilyl chloride in pyridine, following the method used by Long and Burger (3) to prepare a

similar compound. The acetyl group was then removed by refluxing with an ethanol-hydrochloric acid mixture according to the procedure of the above authors.

Experimental Part[1]

4-Dimethylamino-3,5-dibromonitrobenzene. A mixture of 40.7 g. (0.100 mole) of 4-iodo-3,5-dibromonitrobenzene[2], 80 ml. of butanol, and 15 ml. of dimethylamine was heated in a sealed tube at 120-130° for 7 hours. At this temperature a homogeneous solution was obtained. Upon cooling, the product crystallized out in the tube. The tube was opened (no excess pressure), and the contents collected by filtration and washed with methanol. Recrystallization from 96% ethanol yielded 25.3 g. of product as golden orange plates, m.p. 102.5-104°. A second crop weighing 2.2 g. was obtained by concentration of the mother liquors. The total yield was 27.5 g. (85%).

$C_8H_8O_2N_2Br_2$	(324.0)	Calc.	C	29.7	H	2.5	N 8.6
		Found	C	29.8	H	2.5	N 8.5

4-Dimethylamino-3,5-dibromaniline. A suspension of 15.0 g. (0.046 mole) of 4-dimethylamino-3,5-dibromonitrobenzene in 120 ml. of absolute ethanol was hydrogenated at about 60° under 40 lbs. pressure of hydrogen in the Burgess-Parr apparatus, using as catalyst an ethanolic suspension of

[1] Melting points are corrected.
[2] Prepared by J. T. Maynard.

2 g. of Raney nickel previously saturated with hydrogen for 20 min. at 60°. The reduction was complete in a few minutes, but shaking was continued for 15 min. The catalyst was removed by filtration, and the filtrate was then evaporated to dryness under reduced pressure in a bath at 40° to give a quantitative yield of a pale yellow oil which rapidly colored to a reddish violet. It was therefore employed immediately for the next reaction.

4-Acetylamino-4'-dimethylamino-3',5'-dibromobenzenesulfonanilide. The oil obtained from the reduction of 15.0 g. (0.046 mole) of 4-dimethylamino-3,5-dibromonitrobenzene was dissolved in 25 ml. of dry pyridine and coupled with 12.0 g. (0.051 mole) of acetylsulfanilyl chloride in ten portions, allowing the reaction mixture to warm up. The solution was then heated at 60-70° for 80 min. and poured over 200 g. of ice-water slush. The solid was filtered, washed with a large quantity of water, dissolved in 0.5 N sodium hydroxide solution (380 ml.), filtered, and precipitated with conc. hydrochloric acid. The precipitate was then collected, washed with water, and dried in the oven at 90° to give 21.7 g. (95%) of almost colorless product melting at 248.5-250.5°. This material was sufficiently pure for the subsequent hydrolysis. For analysis, a sample was crystallized from ethanol-water in colorless

needles, m.p. 252-253° (decomp.) with darkening from 240°.

$C_{16}H_{17}O_3N_3Br_2S$ (491.2)	Calc.	C	39.1	H	3.5	N 8.6
	Found	C	39.5	H	3.7	N 8.6

4-Amino-4'-dimethylamino-3',5'-dibromobenzenesulfonanilide. A solution of 21.5 g. (0.044 mole) of the acetyl compound in a mixture of 360 ml. of 96% ethanol and 108 ml. of conc. hydrochloric acid was refluxed for one hour. It was then poured into 1100 ml. of water. When the solution was brought to pH 8 with conc. ammonia solution, crystallization occurred. The precipitate was collected, washed, and dried in the oven at 90° to give 18.4 g. of crude product, m.p. 187-190°. Upon recrystallization from 300 ml. of 64% ethanol, 15.5 g. (79%) of almost colorless platelets were obtained, m.p. 193-194.5° at 1°/min.

$C_{14}H_{15}O_2N_3Br_2S$ (449.2)	Calc.	C	37.4	H	3.4	N 9.4
	Found	C	37.3	H	3.3	N 9.4

4-Aminomethyl-3',5'-dibromobenzenesulfonanilide (III)

An attempt was made to prepare 4-cyano-3',5'-dibromobenzenesulfonanilide from I following the procedure used by Miller, et al. (11) to prepare p-cyanobenzenesulfonamide

from sulfanilamide. The method failed because of the insolubility of both I and its diazonium salt.

The required compound was obtained by coupling p-cyanobenzenesulfonyl chloride with 3,5-dibromaniline in pyridine. The product of this reaction was then catalytically reduced using platinum oxide in ethanolic hydrochloric acid, a method described by the above authors (11) to reduce p-cyanobenzenesulfonamide. Attempts to use palladium as the catalyst apparently resulted in reduction of the halogens.

p-Cyanobenzenesulfonyl chloride was prepared by means of a remarkable reaction described by Remsen, et al. (12): p-carboxybenzenesulfonamide, when heated with phosphorus pentachloride, undergoes chlorination followed by rearrangement to the required compound.

Subsequent to the completion of this work, the preparation of the 4-aminomethyl derivative was reported by Kaplan and Leubner (4). The method they used was different, however, and their paper indicates that the compound was obtained by coupling p-acetaminomethylbenzenesulfonyl chloride with 3,5-dibromaniline, followed by acid hydrolysis of the acetyl group. The melting point reported was 1° higher than that found here.

Experimental Part[1]

p-Carboxybenzenesulfonamide. p-Toluenesulfonamide was oxidized with dichromate according to the method described by Remsen (13). The yield was 64% for a run of 0.5 mole. The product melted at 264.5-266° at 0.7°/min. from 230°, instead of decomposing at 280° without melting, as was reported.

p-Cyanobenzenesulfonyl chloride. This compound was prepared following the procedure of Remsen, et al. (12). The product, after distillation, was recrystallized from 60-70° ligroin using a Soxhlet extractor. The yield was 82% for a 0.3 mole run, m.p. 109-111°.

4-Cyano-3',5'-dibromobenzenesulfonanilide. To a solution of 24.0 g. (0.096 mole) of 3,5-dibromaniline in 48 ml. of dry pyridine, 19.6 g. (0.097 mole) of p-cyanobenzenesulfonyl chloride were added in 18 portions. The addition took 20 min. and the temperature did not rise above 35°. The reaction mixture was left at room temperature for 45 min., heated on a steam bath for three hours, and then poured over a mixture of 240 g. of ice, 650 ml. of water, and 96 ml. of conc. hydrochloric acid. After stirring for two hours, the product was filtered, washed with a large quantity of water, and recrystallized by dissolving in 500 ml.

[1] Melting points are corrected.

of boiling 96% ethanol and adding hot water to incipient crystallization (500 ml.). After chilling, the product was collected, washed with water, and dried over phosphorus pentoxide to give 36.9 g. (93%) of colorless plates and flat prisms, m.p. 194.5-195.5° at 0.6°/min. from 180°. The product was analytically pure.

$C_{13}H_8O_2N_2Br_2S$ (416.1)	Calc.	C	37.5	H	1.9	N 6.7
	Found	C	37.3	H	2.0	N 6.7

4-Aminomethyl-3',5'-dibromobenzenesulfonanilide hydrochloride. A suspension of 37.5 g. (0.090 mole) of 4-cyano-3',5'-dibromobenzenesulfonanilide in 920 ml. of absolute ethanol containing 0.112 mole of hydrochloric acid was shaken with 3.0 g. of platinum oxide under atmospheric pressure of hydrogen. The theoretical quantity of hydrogen was absorbed in five hours, and the product was completely dissolved. After filtering off the catalyst, the solvent was removed under reduced pressure, and the residue was extracted with 1200 ml. of boiling water. The solution was filtered from some insoluble solid and oil, and on cooling, deposited large, colorless, flat prisms. These were collected, washed with a large quantity of water, and dried over sodium hydroxide to give 30.4 g., m.p. 271-272° (decomp. with effervescence) at 0.5°/min. from 240°. By concentration of the mother liquors, a second crop weighing 2.7 g. was ob-

tained, to give a total yield of 33.1 g. (78%). A sample recrystallized from water for analysis melted at 273-274° under the conditions recorded above.

$C_{13}H_{12}O_2N_2Br_2S \cdot HCl \cdot H_2O$ (474.6)

Calc.	C	32.9	H	3.2	N 5.9
Found	C	32.8	H	3.4	N 5.9

The free base (4) was obtained by neutralizing a hot solution of the hydrochloride with saturated potassium bicarbonate solution. Recrystallization from absolute ethanol gave colorless, small, flat prisms, m.p. 214.5-215.5° at 0.5°/min. from 205°.

$C_{13}H_{12}O_2N_2Br_2S$ (420.1)

Calc.	C	37.2	H	2.9	N 6.7
Found	C	37.0	H	3.1	N 6.6

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The Resolution of 3-Fluoro-dl-tyrosine, and the Determination of the Toxicity of the Optically Active Isomers

There have been numerous investigations on the possible relationship of fluorine to thyroid activity, but there is little agreement as to how the gland is affected. The synthesis of 3-fluoro-dl-tyrosine by Schiemann and Winkelmüller (1) in 1932 led to some interesting, though complicating, observations.

In 1936, Kraft (2) reported that this compound antagonized the effect of thyroxine on tadpoles, thereby slowing down the increased rate of metamorphosis. Litzka (3) then claimed that 3-fluorotyrosine acted antagonistically to thyroid secretions. Kraft and May (4), Litzka (3), and May (5) have all reported favorable results from the use of 3-fluorotyrosine in treating hyperthyroidism. More recently, Boyer, Evans, and Phillips (6) in this country have studied the physiologic effects of this and other fluorinated amino acids prepared by Dr. Carl Niemann, and have redetermined its toxicity. The results are not in agreement, for the latter workers found that 3-fluorotyrosine was without effect on the basal metabolic rate of the white rat, and that the highly toxic nature of the drug contra-indicated its use as a therapeutic agent. A further interesting phenomenon was observed; namely, that 3-fluoro-tyrosine had an antagonistic action to inorganic fluoride deposition in the bones.

The latter effect of the compound and its highly toxic nature evoke considerable interest in its mode of action. This study was undertaken in order to determine whether this action is a result of the incorporation of the amino acid into some physiologically active polypeptide arrangement by the enzymes of the body. The latent period of six to twelve hours preceding the observable effects of the drug aids such speculation, and if the L-antipode were to exhibit a greater toxicity than the D-antipode, such a mechanism might, indeed, be indicated, in view of the stereoisomeric specificity of proteolytic enzymes.

3-Fluoro-dl-tyrosine was resolved by employing an asymmetric enzymatic synthesis of the L-anilide from the benzoyl derivative. This method of amino acid resolution was first described by Max Bergmann and his coworkers (7,8) and was used to prepare D-glutamic acid and D- and L-phenylalanine. The enzymatic method of resolution is a splendid application of the earlier demonstrations by this group that papain, as well as other proteinases, can perform the synthesis of -CO-NH- linkages (9). Since it was further shown that only the L-form of the amino acid participates in the synthesis, the method is especially useful in this instance, since it simultaneously

establishes the configurational relationships of the optically active isomers.

The optimum pH for enzymatic synthesis with papain-cysteine has been shown to be close to 4.6 (9). This presented a problem, for it was found that below pH 5.8, benzoyl 3-fluoro-dl-tyrosine precipitated from solution. At this pH, the efficiency of the enzyme is very greatly reduced, and a relatively long period was required for the synthesis. Benzoyl 3-fluoro-dl-tyrosine was treated with aniline in the presence of papain-cysteine at pH 5.8. After two weeks of incubation at 40°, the precipitated L-anilide was filtered off; and the filtrate was then acidified and extracted with ethyl acetate to obtain the unreacted D-acid. The benzoyl 3-fluoro-D-tyrosine and benzoyl 3-fluoro-L-tyrosine anilide were then hydrolyzed with hydrochloric acid under conditions which were found by E. Fischer (10) to result in no racemization of L-tyrosine. In this way, the required enantiomorphs were obtained.

Toxicity studies were made employing the procedure described by Boyer, Evans, and Phillips (6). Single subcutaneous injections of solutions of 3-fluorotyrosine hydrochloride were given to rats weighing between 150 and 300 grams. The observable symptoms of toxicity were

identical with those reported: alternate periods of depression and hyperirritability followed by convulsive seizures, and in some cases, by death. Most of the deaths occurred during the first twenty-four hours, but in several instances the period extended to forty-eight hours.

The minimum lethal dose (MLD_{50}) was considered to be that dose which resulted in the death of fifty per cent of the animals to which it was given. Determinations of this minimum lethal dose on mature white rats gave results in good agreement with those obtained by previous workers: 7.5×10^{-5} moles/kg. (15 mg./kg.) as against 6.3×10^{-5} moles/kg. (12.5 mg./kg.) found by both Phillips (6) and Litzka (3). The D-, L-, and dl-forms all exhibited the same MLD_{50}. Unfortunately, this observation does not favor any particular speculation with regard to the mechanism of action of the drug.

Experimental Part

The Resolution of Benzoyl-3-fluoro-dl-tyrosine

Enzymatic Synthesis of the L-anilide.

A solution of benzoyl 3-fluoro-dl-tyrosine[1] was prepared by dissolving 8.75 g. in a warm mixture of 35 ml.

[1] Prepared by Dr. P. L. Nichols, Jr.

of N sodium hydroxide solution and 44 ml. of 2 M sodium acetate and filtering to remove a small quantity of insoluble material. To this was added 88 ml. of 0.1 M citrate buffer, 0.650 g. of cysteine hydrochloride, 5.25 ml. of aniline, and 88 ml. of a papain solution prepared by dissolving 0.90 g. of purified papain[2] in 100 ml. of 0.05 M citrate buffer and filtering. The mixture was then diluted with 170 ml. of water. The pH of this solution was 6.0. Adjustment to pH 5.8 was made by adding 1.0 ml. of 50 per cent acetic acid. The reaction mixture was placed in an oven at 40° for seven days, the pH being lowered on the second and third days by the addition of 1.0 ml. of 50 per cent acetic acid. The precipitate was then filtered off and washed with cold water and 50 per cent ethanol. The weight of dried anilide was 3.25 g., m. p. 194-196.5° (decomp.). The filtrate was adjusted to pH 5.5 and kept at 40° for another week. An additional crop of 1.70 g. melting at 192-195.5° (decomp.) was obtained. The weight of benzoyl 3-fluoro-L-tyrosine anilide (4.95 g.) corresponded to 91 per cent of theory. Attempts to purify the material were not very successful. The approximate specific rotation of the crude anilide was +12.4° (c = 2 per cent in pyridine) at 30°.

[2] Prep. by Dr. P. L. Nichols, Jr. according to the method of Bergmann and Fraenkel-Conrat (9).

Recovery of the Benzoyl 3-fluoro-D-tyrosine.

The filtrate containing the benzoyl 3-fluoro-D-tyrosine was acidified to Congo red with concentrated hydrochloric acid, and extracted five times with a total volume of one liter of ethyl acetate. After drying over sodium sulfate, the ethyl acetate was removed under reduced pressure. The resulting brown oil partially crystallized on prolonged evaporation.

Hydrolysis of the Optically Active Benzoyl Compounds.

A suspension of 4.95 g. of benzoyl 3-fluoro-L-tyrosine anilide in 200 ml. of 10 per cent hydrochloric acid was heated at 100° for 18 hours. The reaction mixture was cooled, and the precipitated benzoic acid was filtered off. The remaining benzoic acid was removed from the solution by two extractions with ether. The aqueous phase was then concentrated under reduced pressure to a volume of 50 ml. and brought to neutrality by the addition of solid sodium acetate. Upon the addition of ether to dissolve the separated aniline, the product crystallized. The ether was decanted and the product filtered and washed well with water. After two recrystallizations from water, the specific rotation was -5.7°, m.p. 278-9° (decomp.) at 5°/min. from 265°. The yield was 1.2 g.

Anal. $C_9H_{10}O_3NF$ (199.2) Calc. C 54.3 H 5.1 N 7.0

Found C 54.5 H 5.2 N 6.9

Spec. Rot. $[\alpha]_D^{26^\circ} = \frac{-0.29 \times 1.95}{1 \times 0.1000} = -5.7^\circ$ (in 4% HCl)

The benzoyl 3-fluoro-D-tyrosine was treated in the same manner. The brown oil was heated at 100° with 200 ml. of 10 per cent hydrochloric acid for 18 hours. After cooling, the benzoic acid was extracted with ether and the aqueous phase concentrated under reduced pressure. Upon neutralizing with sodium acetate, removing the separated aniline with ether, filtering, and drying, 2.2 g. of material with a specific rotation of +2.9° was obtained. This was dissolved in the minimum quantity of boiling water, cooled, and the precipitate filtered off. The specific rotation of the precipitate was +1.4° showing that the dl-form is considerably less soluble than the enantiomorph. The mother liquors were evaporated to dryness and the residual solid recrystallized from water to give 0.9 g. with a specific rotation of +5.7°, m.p. 279-280° (decomp.) at 5°/min. from 265°.

Anal. $C_9H_{10}O_3NF$ (199.2) Calc. C 54.3 H 5.1 N 7.0

Found C 54.3 H 5.1 N 6.9

Spec. Rot. $[\alpha]_D^{26^\circ} = \frac{+0.29 \times 1.95}{1 \times 0.1000} = +5.7^\circ$ (in 4% HCl)

Melting points are corrected.

Toxicity Determinations

Preliminary experiments with younger rats (80 to 150 grams), for which the toxicity was found to be more acute than with mature rats (6), indicated that the minimum lethal dose for 3-fluoro-L-tyrosine was the same as that for the racemic form (Table 1).

Table 1

Toxicity of 3-fluoro-L-tyrosine for immature rats

Dose moles/kg. x 10^5	Dose mg./kg.	Number of rats	Number died
3.0	6.0	6	0
3.5	7.0	6	0
4.0	8.0	6	0
4.5	9.0	6	0
5.0	10.0	4	0
6.3	12.5	4	2
7.5	15.0	4	4

Further preliminary experiments were made to compare

the effects of the D-, L-, and dl-forms. Rats weighing between 90 and 160 grams were used (Table 2).

Table 2

Comparison of the effect of D-, L-, and dl-forms of 3-fluorotyrosine on immature rats

Substance	Dose moles/kg. x 10^5	Number of rats	Number died
3-fluoro-D-tyrosine	6.3	6	2
3-fluoro-L-tyrosine	6.3	6	3
3-fluoro-dl-tyrosine	6.3	6	2

A final comparison of activity of the enantiomorphs and the racemate was made at two levels: 6.3 and 7.5 x 10^{-5} moles/kg. These experiments were done with mature white rats (weighing between 150 and 300 grams) and were run simultaneously. The results (Table 3) do not indicate any difference in toxicity of the three forms.

Table 3

Comparison of the toxicity of
3-fluoro-D-, L-, and dl-tyrosine
for mature rats

Substance	Dose moles/kg. x 10^5	Number of rats	Number died
3-fluoro-D-tyrosine	6.3	8	0
	7.5	8	4
3-fluoro-L-tyrosine	6.3	8	0
	7.5	8	4
3-fluoro-dl-tyrosine	6.3	8	0
	7.5	8	6

I wish to express my gratitude to Mary L. Sease for her valuable assistance in these toxicity determinations.

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The Synthesis of p-Arsono-dl-phenylalanine

P-Arsono-dl-phenylalinine was to be employed in order to gain some further insight into the problem of the extent to which amino acids may be modified without disturbing their ability to serve as substrates for proteolytic enzymes.

The proposed synthesis was as follows:

N-Acetylphenylalanine
↓
p-Nitro-N-acetylphenylalanine
↓
p-Amino-N-acetylphenylalanine
↓
p-Arsono-N-acetylphenylalanine
↓
p-Arsonophenylalanine

N-Acetylphenylalanine, prepared from aceturic acid and benzaldehyde according to the directions of Herbst and Shemin (1), was nitrated at 0° in sulfuric acid following the method employed by Erlenmeyer and Lipp (2) to nitrate phenylalanine. The yield (44%) was found to be reduced at room temperature, while the use of acetic anhydride as the solvent gave extremely poor results. To confirm the para position for the substitution, a sample was oxidized with permanganate to p-nitrobenzoic acid. A large quantity of byproduct obtained in the nitration was found to melt at 136-138° after recrystallization from

water. This compound was not characterized further, but it is believed to be the ortho isomer.

The nitrophenylalanine was catalytically reduced in ethanol using platinum oxide. The crystalline amine was isolable only when the nitro compound was exceptionally pure. In other cases, the product was obtained as a red oil.

Attempts to introduce the arsonic acid group by means of the Bart reaction (3) or its several modifications were not very satisfactory, and the water solubility of the product made isolation difficult. It must be noted, however, that in view of the isolation difficulties, most of the experiments were done with quantities insufficiently large (1.5-3.5 g.) to make the results unequivocal.

Hydrolysis of a small quantity of the N-acetyl derivative with N hydrochloric acid gave a compound which analyzed approximately for the monohydrate of the disodium salt of p-arsonophenylalanine after precipitation from aqueous solution at neutral pH with ethanol. Further work was discontinued pending the return to peacetime pursuits.

It seems certain that a considerable improvement in the above synthesis could be effected by starting with hippuric acid and p-nitrobenzaldehyde, thereby avoiding the poor yield obtained in the nitration and decreasing

the water solubility of the intermediates. The use of p-aminobenzaldehyde might also be considered in view of a recent description of its preparation from p-nitrotoluene in 75 per cent yield (4).

Experimental Part[1]

N-Acetylphenylalanine (1). A total of 122 g. of this compound was prepared according to the directions given in Organic Syntheses.

p-Nitro-N-acetylphenylalanine. To a solution of 25.0 g. (0.121 mole) of N-acetylphenylalanine in 62 ml. of conc. sulfuric acid, 6.0 ml. of conc. nitric acid (sp.g. 1.5) was added dropwise over a 20 min. period, keeping the temperature at -10 to +5°. The solution was allowed to stand at 0° for three hours and placed in the cold room overnight. It was then poured over 600 g. of cracked ice. The product, which crystallized on standing in the cold room, was filtered, washed well with water, and recrystallized from 500 ml. of water to give 13.4 g. (44%), m.p. 194-196°. After recrystallization from ethyl acetate-ethanol (6:1) the melting point was 198.5-200.5°. An analytical sample melted at 200.5-201.5°.

$C_{11}H_{12}O_5N_2$	(252.2)	Calc.	C	52.4	H	4.8	N 11.1
		Found	C	52.3	H	4.5	N 11.0

[1] Melting points are corrected.

A sample of 0.5 g. of the above compound was refluxed for three hours with 40 ml. of 5% potassium permanganate containing 0.5 g. of sodium hydroxide. The acid was isolated in the usual manner and recrystallized from water to give colorless needles, m.p. 240.5-242°. The literature gives the melting point of p-nitrobenzoic acid as 242°, whereas the ortho and meta substituted acids melt at 146° and 140° respectively.

p-Amino-N-acetylphenylalanine. A solution of 10.5 g. (0.042 mole) of p-nitro-N-acetylphenylalanine in 200 ml. of 96% ethanol was shaken in the Burgess-Parr apparatus with 0.4 g. of platinum oxide under three atm. pressure of hydrogen. Hydrogenation was complete in 20 min. The catalyst was filtered off and the solvent removed at reduced pressure under nitrogen. The product was recrystallized from 200 ml. of 96% ethanol, filtered, and dried over sulfuric acid to give 6.5 g. (70%) of almost colorless needles, m.p. 176.5-179.5°.

$C_{11}H_{14}O_3N_2$	(222.2)	Calc.	C 59.5	H 6.4	N 12.6
		Found	C 59.5	H 6.6	N 12.3

The N-benzoyl derivative, prepared in the usual manner with benzoyl chloride in alkaline solution, melted at 241.5-243° after two recrystallizations from water.

$C_{18}H_{18}O_4N_2$	(326.3)	Calc.	C 66.3	H 5.6	N 8.6
		Found	C 66.5	H 5.6	N 8.4

p-Arsono-N-acetylphenylalanine. To a solution of 13.3 g. (0.060 mole) of the above amine and 12.2 ml. of conc. hydrochloric acid in 40 ml. of ice water, a solution of 4.6 g. of sodium nitrite in 10 ml. of water was carefully added (to a starch-potassium iodide end-point). The temperature was kept at 5-8° by regulating the rate of addition of the nitrite solution and occasionally adding powdered dry ice. The solution was allowed to stand at 0-5° for 30 min. and was then poured rapidly and with vigorous mechanical stirring into a solution (at 0°) containing 9.55 g. (0.074 mole) of sodium arsenite (20% excess), 1.0 ml. of 3 M copper sulfate, and 0.3 g. of cuprous chloride. Simultaneously, 30 ml. of 5 N sodium hydroxide (0.150 mole) was added dropwise. The foaming was moderate; the pH was 7-8 (Universal paper); the temperature rose to 18° but was caused to drop by the addition of powdered dry ice. The reaction mixture was stirred at 5-10° for 30 min. and then for 1½ hrs. while allowing it to come to room temperature. After standing in the cold room overnight, it was warmed to 60° until nitrogen evolution ceased, allowed to cool, and suction filtered. The slightly alkaline solution was then neutralized with acetic acid and concentrated at atmospheric pressure to 75 ml. It was treated with 1 g. of norite, filtered with suction, and the carbon residue was washed with hot

water. The combined filtrate and washings (100 ml.) were carefully acidified to Congo red by dropwise addition of 10 ml. of conc. hydrochloric acid. A black-brown oil precipitated which crystallized after standing in the cold room several days. It was partially purified by dissolving in potassium bicarbonate and precipitating with acid, and by washing with ethyl acetate to remove a small quantity of contaminating oil. The yield was 7.6 g. (38%) of a light brown powder. This material, which did not melt below 300°, contained some arsenic trioxide.

A small quantity of pure product was obtained in poor yield by the Ruddy, Starkey, and Hartung (5) modification of the Bart reaction. Purification was effected by precipitation from 90% aqueous dioxane by addition of ethyl acetate. This method of purification could not be repeated with material obtained from other runs. The product was a colorless powder, soluble in water, and decomposing without melting at 280-290°. It left no residue when ashed and gave a negative ninhydrin test.

$C_{11}H_{14}O_6NAs$	(331.4)	Calc.	C	39.9	H	4.3	N 4.2
		Found	C	40.2	H	4.6	N 4.0

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A Preliminary Study of the Proteins in Some Invertebrate Body Fluids

This report contains a résumé of the results of research performed in connection with a minor in the department of Immunochemistry.

The most recent literature on invertebrate bloods reports that hemocyanin is the only protein present in Crustacean serum (1, 2). In a review of the literature on this protein, Webb (3) also states that hemocyanin is the sole protein of most Molluscan bloods. However, the techniques employed by these and previous investigators are not sufficiently refined to prove the absence of small quantities of non-hemocyanin proteins. There is a widespread occurrence of natural heteroagglutinins in invertebrate bloods, suggesting the presence of other proteins as well as offering a method for differentiating the fractions obtained in any attempted separation.

The first investigation of the relation of these heteroagglutinins to blood proteins was made with the blood of the spiny lobster (Panulirus Interruptus) by Scheer and Tyler (4). In that study, the non-hemocyanin proteins were separated from hemocyanin by dialysis of the plasma or serum against acetate buffer. These proteins were again fractionated by means of ammonium sulfate into fibrinogen and another fraction. In this way three easily separable protein fractions were obtained: hemocyanin, fibrinogen, and a non-fibrinogen fraction. The heteroagglutinin activity was found to be associated

approximately equally with the two non-hemocyanin fractions, while the hemocyanin showed no such property.

In this research, the above investigation of spiny lobster blood was amplified with an electrophoretic study of the three protein fractions; the study of invertebrates was extended to another Crustacean, the crab (Cancer Anthonyi), and to the phyla Echinodermata (the sea cucumber, Stichopus Californicus) and Mollusca (the green abalone, Haliotis Fulgens).

The techniques employed for collecting and fractionating the blood, and for titrating the heteroagglutinin were the same as those described by Scheer and Tyler (4).

An electrophoretic study was made of lobster plasma (I), serum (II), a solution of the non-hemocyanin proteins (III), a solution of the fraction of the latter precipitated by 1/3 saturated ammonium sulfate (IV), and a solution of the remaining fraction soluble in 2/5 saturated ammonium sulfate.

The preponderance of hemocyanin in both plasma and serum is sufficient to mask the existence of small quantities of other components. Hemocyanin is the most mobile protein present. The pattern of solution III was the most significant, and it revealed two distinct proteins: one with a mobility only slightly less than that of hemocyanin, and

another, larger component whose mobility corresponded to that of the second component in the plasma pattern (I). This latter protein is the fibrinogen. The patterns of solutions IV and V showed the fractionation of the components of III, plus another very small component which may have been an artifact. These results confirmed the existence of at least three protein constituents in lobster blood.

The heteroagglutinin activity was found to be almost completely associated with the fibrinogen fraction (IV), in contrast to previous observations (4). The fact that fraction V was collected at 2/5 ammonium sulfate saturation whereas Scheer and Tyler made their separation at only 1/4 saturation may explain this difference.

The chemical fractionation of crab blood, carried out in the same manner as for lobster blood, showed some differences from the latter. Separation of the non-hemocyanin proteins from hemocyanin was not as clean cut, inasmuch as the precipitation of all proteins during dialysis against acetate buffer was much more rapid.

Crab blood heteroagglutinins were found to be active against sperm suspensions from Lytechinus which was there-

fore used as the test antigen.

Ammonium sulfate precipitated the heteroagglutinin activity completely at 2/5 saturation. This result is noteworthy when compared with the similar observation in the lobster blood fractionation. There this level of ammonium sulfate saturation precipitated the fibrinogen with which the agglutinin activity is intimately associated. However, fibrinogen is not present in crab plasma, and it is interesting to speculate on whether the lobster agglutinin activity is really a function of the fibrinogen, or whether the very small component observable in the electrophoresis pattern of the fibrinogen fraction (which was previously dismissed as a possible artifact) is of significance in this regard. Above 2/5 saturation with ammonium sulfate, more protein, totally inactive, was precipitated.

This fractionation showed that there are at least two, and possibly three, protein constituents in crab serum.

The blood of the sea cucumber is much simpler constitutionally than those of either the crab or the lobster, for there is no respiratory protein present. However, heteroagglutinin activity against Urechis sperm is marked.

The concentration of protein in the blood was so small (ca. 0.02 per cent) that precipitation could be effected only at ammonium sulfate concentrations close to saturation. When the fluid was previously concentrated to 1/3 volume, 90 per cent of the protein present was precipitated by saturated ammonium sulfate. This precipitate was found to contain all the agglutinin activity. It seems reasonable to suppose, then, that the sea cucumber body fluid contains only one protein constituent.

Abalone blood was found to have agglutinins with marked activity against both Lytechinus and S. Purpuratus sperm suspensions.

A preliminary fractionation by dialysis against tap water indicated that the agglutinin activity was not associated with the hemocyanin, but with a grey-colored, gelatinous material which was precipitated. Inasmuch as the heteroagglutinins are probably protein in nature, it would seem safe to conclude that abalone blood contains more than one protein constituent, and that the heteroagglutinin activity is a property of the non-hemocyanin fraction.

I am indebted to Dr. B. T. Scheer, Dr. Dan H. Campbell, and Dr. S. M. Swingle for considerable assistance with this study.

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4. Scheer and Tyler: Unpublished work (1944)

   The Relation of Natural Hetero-agglutinins to Blood Proteins in the Invertebrates.

Summary

I The syntheses of two series of compounds, benzenesulfonanilides and carbinolamines related to quinine, are described. These compounds were prepared as potential antimalarials.

II The enzymatic resolution of N-benzoyl 3-fluoro-dl-tyrosine and the preparation of 3-fluoro-D- and L-tyrosine are described. A study of the acute toxicity of the optical isomers is also reported.

III The synthesis of p-arsono-N-acetylphenylalanine is described.

IV The chemical fractionation of the protein constituents of Panulirus Interruptus (spiny lobster) blood was repeated, and an electrophoretic study of the fractions, confirming the presence of at least three separate proteins, is described. Preliminary fractionations of the body fluids of Cancer Anthonyi (crab), Stichopus Californicus (sea cucumber), and Haliotis Fulgens (green abalone) are also reported.

Acknowledgments

I wish to express my sincere appreciation to all the members of the staff who assisted me with the work herein described, especially Dr. Joseph B. Koepfli and Professors Carl Niemann and Dan Campbell.

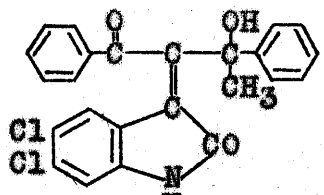
I am also indebted to my colleagues in the laboratory for their suggestions and generous cooperation with many of the problems.

I am especially grateful to my wife, Edith, for aid in the preparation of this thesis and for considerable assistance with several phases of the work.

The microanalyses are by Dr. Gertrude Oppenheimer and her staff.

Propositions

1. The by-product obtained in the reaction of 5,6-dichloroisatin with excess acetophenone under Pfitzinger conditions (thesis, p. 33) probably has the following structure:



Sumpter: Chem. Revs., 34, 393 (1944)

2. Methylamine is a potentially useful agent for cleaving N-alkylphthalimides and has several advantages over present reagents.

J. F. Mead: Private communication
Ristenpart: Ber., 29, 2530 (1896)

3. The contention of Karrer and Epprecht that Ackermann's method of preparing benzenesulfonyl guanidine leads instead to guanidinium benzenesulfonate is not wholly justified. The previous detailed study of Clarke and Gillespie indicates that both compounds are formed, albeit the salt is in greater yield. According to these reports, Hess and Sullivan's preparations of bis-(β-naphthalenesulfonyl)-guanidine and β-naphthalenesulfonyl-N-methylguanidine are in error.

Karrer and Epprecht: Helv., 24, 310 (1941)
Ackermann: Zeit. physiol. Chem., 47, 366 (1906)
Clarke and Gillespie: J.A.C.S., 54, 1964 (1932)
Hess and Sullivan: J.A.C.S., 57, 2331 (1935)

4. Sulfonylguanidines can be prepared by heating the sodium salt of a sulfonic acid with guanidine hydrochloride. This method may be generally applicable to the preparation of sulfonamides.

5. The melting points of organic compounds, as commonly determined, are dependent on both the rate of heating and the temperature at which heating is begun, and this dependence is even more marked where decomposition simultaneously occurs. Such decomposition points should

be reported with at least this additional information concerning heating procedure if they are to be useful as identification criteria.

6. In order to explain the fact that in the ethylation of benzene with ethylene and aluminum chloride all the diethylbenzene formed is 1,4 and all the triethylbenzene formed is 1,3,5, Sisido proposes the following steps in the alkylation:

ethylbenzene → 1,3-diethylbenzene → 1,3,5-tri ethylbenzene, followed by rearrangement of the 1,3- to the 1,4-diethylbenzene.

There are several reasons for considering the following mechanism superior:

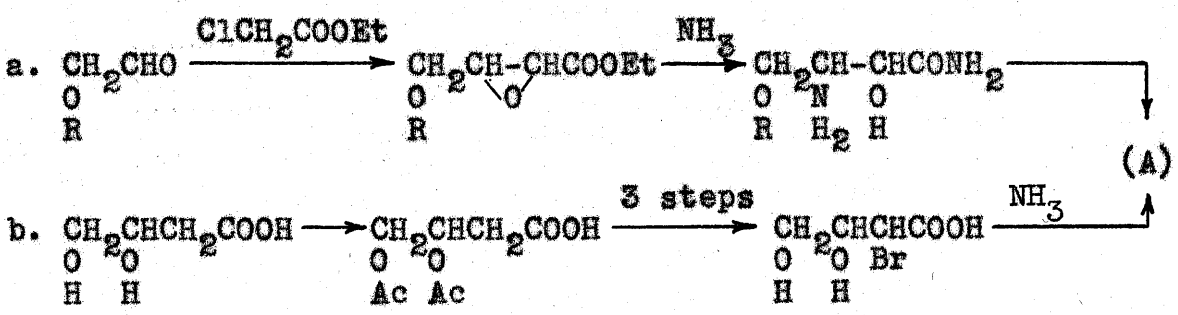
ethylbenzene → 1,4-diethylbenzene → 1,2,4-tri ethylbenzene, followed by rearrangement of the 1,2,4- to the 1,3,5-triethylbenzene.

Sisido: J. Soc. Chem. Ind., Japan, 44, Suppl. binding 104 (1941). Chem. Zentr. (1943), I, 1110. See C.A. 38, 3624 (1944)
Baddeley and Kenner: J. Chem. Soc., (1935) p. 303
Price and Ciskowski: J.A.C.S., 60, 2499 (1938)

7. The preparation of histidyl peptides by the Bergmann method is limited by the extremely poor yields obtained in carbobenzoxylating histidine. This yield may be increased considerably by acylating the ester of histidine.

Bergmann and Zervas: Ber., 65, 1192 (1932)

8. The following methods of synthesis of 1,3-dihydroxy-2-amino-n-butyric acid (A) are proposed:



where R is $C_6H_5CH_2$ and Ac is CH_3CO

9. The heteroagglutinin activity of invertebrate bloods cannot be attributed to hemocyanin; therefore, the existence of such activity in Molluscan bloods indicates the presence of at least two protein constituents.

10. It has been stated by Maluf that calcium is unnecessary for fibrin formation in arthropod bloods. This statement is contradicted by experiment, and the necessity of calcium ion for fibrin formation reveals a close similarity in this phase of the blood-clotting processes of both arthropods and vertebrates.
    Maluf: Quat. Rev. Biol., 14, 152 (1939)
    "The Blood of Arthropods".

11. A course in advanced preparations and technique should be a prerequisite to the undertaking of research by graduate students in organic chemistry. Such a course should be flexible, and its duration should be dependent upon the previous training as well as the progress of the individual student.