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Thesis by

James Franklyn Mead

In Partial Fulfillment of the Requirements

for the Degree of Doctor of Philosophy

California Institute of Technology

Pasadena, 1942

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Acknowledgment

I wish to thank Professor Carl Niemann for his patient and helpful instruction of the past four years. Without his suggestions and criticisms this work could not have been accomplished.

Part I

The Benzylation of Carbohydrates

In the determination of the structure of the polysaccharides, the main method used so far has been that developed by Irvine, Haworth, and co-workers. It consists of complete methylation of the polysaccharide, followed by hydrolysis and identification of the methyl hexoses so obtained. This method has been very fruitful, but has suffered from several faults: First, there is often great difficulty in crystallizing the trimethyl sugars so obtained as hydrolysis products of the polysaccharides. This is especially true if even very small traces of impurities are present, which is usually the case. Some of the trimethyl glucoses have been obtained only as syrups, recognizable only by the rotations, which are also greatly affected by small amounts of impurities[1]. Furthermore, the structure of the original polysaccharide is still not certain even after the trimethyl glucose has been identified with certainty, since it is not evident from the trimethyl monose just on which carbon the interglucosidic and on which the intraglucosidic bridges were attached. This difficulty has been largely dealt with by the use of various degradation methods, as that of Zemplén[2].

As a solution to both these difficulties, Dr. Niemann proposed the complete benzylation of the polysaccharides, followed by methylation and identification of the methylated sugars after removal of the benzyl residues by catalytic hydrogenation. The determination should proceed as follows:

First, the complete benzylation of the polysaccharide should be

assured. Then, with methanolic hydrogen chloride, the interglucosidic oxygen bridges would be ruptured, and any change in the intraglucosidic bridge prevented by methylation of the two positions involved. There would thus be obtained dimethyl benzyl monoses. On catalytic hydrogenation of these would be obtained dimethyl monoses, the methyl groups being attached at the points of attachment of the former interglucosidic oxygen bridges. The intramolecular bridge should be in the same position it occupied in the intact polysaccharide.

As a start in the synthesis of the benzylated polysaccharides, complete benzylation of glucose was attempted using different methods with varying success until finally a method based on that of Zemplén and co-workers[3] gave the desired product. At this point the problem was dropped temporarily for work with Dr. James English, Jr. as described in Part II.

Experimental Part

I. Preparation of Pentabenzyl Glucose.

Ten methods of benzylation were tried with varying success. In some cases no product could be isolated. In others a syrup was obtained. Finally the method of Zemplén et. al. was applied.

α-Methyl-d-Glucoside.--- Following the method of Patterson and Robinson[4], 200 g. of α-methyl-d-glucoside, m.p. 162-163°, was obtained from 500 g. of glucose.

Tetracetyl-α-Methyl-d-Glucoside.--- Following the method of Koenig and

Knorr[5], from 100 g. of the above α-methyl-d-glucoside was obtained 80 g. of the tetracetyl compound of m.p. 101-102°.

Tetrabenzyl-α-Methyl-d-Glucoside.----A three-neck flask was fitted with a thermometer and a mercury-seal stirrer, while on the third neck an erlenmeyer flask was attached by means of a Gooch rubber. This permitted the slow addition of solid to the reaction mixture without exposure to air or moisture.

Into this reaction vessel were introduced 125 cc. of benzyl chloride and 18 g. of the above tetracetyl methyl glucoside. The solution was stirred at 95-100° on the water-bath while 45 g. of powdered potassium hydroxide was added during the course of three hours. The mixture was then stirred two additional hours, cooled, and poured into cold water. The mixture was extracted with chloroform and the chloroform solution washed with water until neutral, and then steam distilled until no more biphase material came over. On cooling, 17 g. of syrupy residue separated. This could not be crystallized by any means tried, and so it was discarded. However, the specific rotation was taken.

$$[\alpha]_D^{23} = 52.3 \quad (CHCl_3)$$

Tetracetyl-β-Benzyl-d-Glucoside[6].----20 grams of acetobromoglucose prepared after Dr. C. E. Redeman's modification of the method given by Gatterman[7], was shaken with 27 g. of dry ether, 100 g. of benzyl alcohol, and 14 g. of silver oxide during three hours, or until a small sample gave no precipitate with silver nitrate solution. After steam distillation at reduced

pressure of the benzyl alcohol, a solid was obtained, which crystallized from 50% ethanol in water to give 16 g. of the product, of m.p. 96-100°. This was used in the next step without further purification.

Pentabenzyl Glucose.---16 grams of the above prepared tetracetyl-β-benzyl-d-glucoside was treated, as described before in the preparation of the tetrabenzyl compound, with 155 ml. of benzyl chloride and 50 g. of powdered potassium hydroxide. The reaction mixture was not steam distilled, but was washed with water and evaporated at 20 mm. on the water-bath. It was then distilled at 5 mm. and 80° until the residue crystallized on cooling. On recrystallization from methyl alcohol was obtained 15 g. of colorless crystals of m.p. 86-87°, as reported in the literature[3].

From the few results obtained in this problem, it is still evident that for the preparation of completely benzylated polysaccharides a convenient method should be that of the reaction of the completely acetylated compounds with benzyl chloride in the presence of anhydrous potassium hydroxide. This method has not been tried on these compounds as yet, but the indications are that it will have considerable success when it is.

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Part II

The Preparation of Fluorinated Analogues of Tyrosine and Thyronine

It has been shown by many investigators that inorganic fluorine has some effect on the thyroid gland; but just what this effect is and how it manifests itself is not clear.

Goldemberg[1] found that sodium fluoride caused a decrease in the basal metabolism rate in rats and used the salt in the treatment of human hyperthyroidism.

Evans and Phillips[2], however, found no correlation between the fluorine content of the thyroid gland and the basal metabolic rate of human patients. The same co-workers[3,4] showd that administration of inorganic fluoride definitely increased the toxicity of desiccated thyroid for the guinea-pig, rat, and chick.

Many other workers have obtained various results using inorganic fluorine; and more recently Kraft[5] showed that 3-fluorotyrosine slowed up the increased rate of metamorphosis produced in tadpoles by administration of thyroxine. Kraft and May[6], Litzka[7], and May[8] used this compound in the treatment of hyperthyroidism of human patients.

In order to carry these investigations farther we undertook a co-operative project with Dr. Paul Phillips of the University of Wisconsin, in which we supplied a number of fluorinated amino acids for his pharmacological work. The amino acids prepared were first 3-fluoro-dl-tyrosine, 3-fluoro-5-iodo-dl-tyrosine, 3,5-difluoro-dl-tyrosine, and 3-fluoro-dl-phenylalanine. The preparation of these compounds is described in this paper, and has been published in a recent Journal[9]. The pharmacological

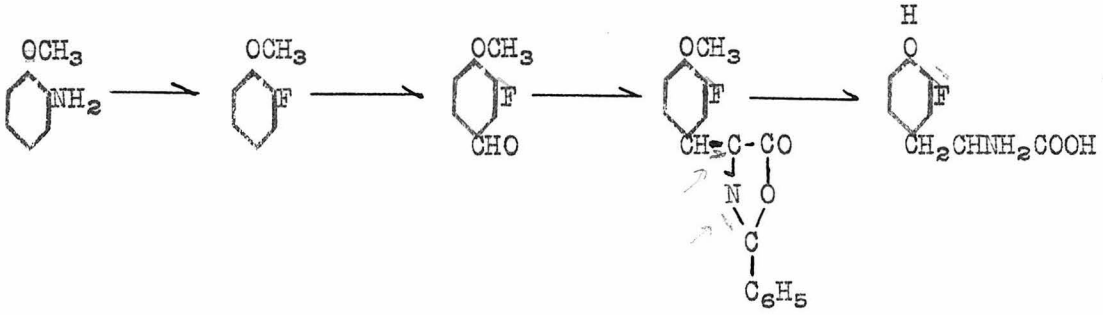
studies have also been published[10]. In these, Phillips and co-workers investigated the toxicity of the compounds, finding that the order was 3-fluorotyrosine > 3-fluorophenylalanine > 3-fluoro-5-iodo-tyrosine > 3,5-diflurotyrosine. The toxic effects were described; and the inhibition of growth in young rats by very small doses of 3-fluorotyrosine, as compared with fairly large amounts of sodium fluoride, was reported.

In a second paper[11] we reported the preparation of some of the corresponding thyroxine analogues, 3'-fluoro-dl-thyronine, 3'-fluoro-3,5-diiodo-dl-thyronine, and 3'-fluoro-5-iodo-3,5-diiodo-dl-thyronine; and in a third paper[12] that of 3',5'-difluoro-dl-thyronine and of 3,5-diiodo-3',5-difluoro-dl-thyronine. The pharmacological investigation of these compounds has not as yet been reported.

Experimental Part

I. The Synthesis of 3-Fluoro-dl-Tyrosine.

This preparation was carried out according to the method of Schiemann and Winkelmuller[13], and followed the scheme:



O-Fluoroanisole[14,15].---To 2.60 kg. of freshly distilled o-anisidine in 5.2 ℓ of concd. hydrochloric acid was added 1.53 kg. of sodium nitrite in 2 ℓ. of water. Throughout the above and succeeding operation the reaction

mixture was stirred vigorously, and the temperature was not allowed to rise above 0°. Five liters of fluoroboric acid, prepared from 4.05 ℓ. of technical hydrofluoric acid and 1.86 kg. of boric acid[16], was added to the clear solution of the diazotized amine, and the mixture was maintained at -10° for one hour. The precipitate was recovered, washed successively with water, ethanol, and ether, and dried *in vacuo* over sulfuric acid. The yield of diazonium fluoborate was 2.7 kg. or 57.5% of the theoretical amount. The diazonium salt was then decomposed in 540 g. portions, in a manner identical with that described in Organic Syntheses[16]. The crude fluoroanisole was taken up in ether, the ethereal solution was washed successively with dilute sodium hydroxide and water and finally dried over sodium sulfate. Fractional distillation gave 820 g. of o-fluoroanisole, b.p. 69-70° (26 mm.), a yield of 53.5% from the diazonium fluoborate or an over-all yield of 30.8% from o-anisidine. In later experiments, using technical sodium fluoborate and crystallizing the mixture at -20° an over-all yield of 64% was obtained.

Anal. Calcd. for C_7H_7OF (126): C, 66.7; H, 5.6. Found: C, 66.8; H, 5.8.

3-Fluoroanisaldehyde[13].--- Twenty grams of o-fluoroanisole was added to 27 g. of zinc cyanide[17] suspended in 100 ml. of benzene. After the solution was saturated with hydrogen chloride, 24 g. of aluminum chloride was added, and the reaction mixture was heated at 40-50° for four hours. The complex, after standing overnight, was decomposed by refluxing with 10%

hydrochloric acid, and the crude aldehyde was isolated as described in the above reference. Fractional distillation gave 6.5 g. of o-fluoranisole and 7.0 g. of 3-fluoroanisaldehyde, m.p. 29-30°. The yield of aldehyde was therefore 29% of the theoretical amount, based on the original starting material, or 42% on the basis of o-fluoroanisole consumed.

This yield could not be improved although a similar reaction with anisole as the starting material gave a 95% yield of anisaldehyde.

Anal. Calcd. for $C_8H_7O_2F$ (154): C, 62.4; H, 4.5. Found: C, 62.4; H, 4.6.

4-(3'-fluoro-4'-methoxybenzal)-2-phenyl-oxazolone-5[13].--- Six grams of 3-fluoroanisaldehyde was condensed with hippuric acid in the presence of acetic anhydride and sodium acetate[13,18] and 11 g. (95%) of the above azlactone, m.p. 207° (corr.), was obtained.

3-Fluoro-dl-Tyrosine[13].--- Thirty grams of the above azlactone gave, on reduction and hydrolysis with hydriodic acid (d. 1.7), red phosphorus, and acetic anhydride, 10.3 g. of 3-fluoro-dl-tyrosine, dec. pt. 275-278°, with rapid heating. The yield of amino acid from 3-fluoroanisaldehyde was 49% of the theoretical amount.

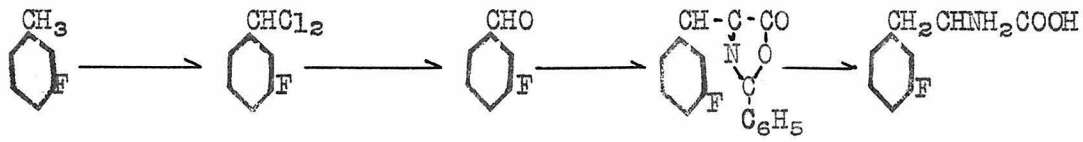
Anal. Calcd. for $C_9H_{10}O_3NF$ (199): C, 54.3; H, 5.0; N, 7.0. Found: C, 54.4; H, 5.2; N, 6.9.

3-Fluoro-5-Iodo-dl-Tyrosine.--- To 7 g. of 3-fluorotyrosine in 8 N ammonium hydroxide solution was added 9 g. of iodine dissolved in the minimum amount of potassium iodide solution, and the mixture was allowed to stand overnight.

The crystalline precipitate was then recovered by filtration, dissolved in dilute alkali, and reprecipitated by the careful addition of acid. The mother liquors were concentrated in vacuo to remove the excess ammonia and were carefully acidified, whereupon a further quantity of 3-fluoro-5-iodo-dl-tyrosine was obtained. After recrystallization from 50% ethanol the compound melted with decomposition at 192°. The yield was 5.4 g. or 47% of the theoretical amount.

Anal. Calcd. for $C_9H_9O_3NFI$ (324.9): C, 32.9; H, 2.8; N, 4.3. Found: C, 32.9; H, 3.1; N, 4.1.

II. The Synthesis of 3-Fluoro-dl-Phenylalanine[19].



m-Fluoro Benzalchloride.--- Fifty-five grams of m-fluorotoluene was refluxed with 5 g. of phosphorus pentachloride while a stream of chlorine was bubbled through the solution until the temperature reached 191°. This corresponded to the addition of 35 g. of chlorine, or one mole. The product, 85 g., was distilled, giving 75 g. of pure m-fluorobenzal-chloride of b.p. 85° at 18 mm.

m-Fluorobenzaldehyde.--- The above prepared 75 g. of m-fluorobenzalchloride was refluxed during four hours with 500 ml. of water and 140 g. of pure

calcium carbonate, while a stream of carbon dioxide was passed into the mixture. Steam distillation gave 40 g. (75%) of the aldehyde of b.p. 73° at 18 mm. On acidification of the filtrate from the steam distillation, 2.5 g. of m-fluoro benzoic acid, of m.p. 120-121°, was obtained.

4-(3-Fluoro Benzal)-2-Phenyl-Oxazolone-(5).--- Thirty-five grams of the above aldehyde was treated in the usual manner with 54 g. of hippuric acid, 25 g. of anhydrous sodium acetate, and 190 ml. of acetic anhydride to give 36 g. (60%) of the above azlactone of m.p. 152-153°.

3-Fluoro-dl-Phenylalanine.---- Thirty grams of the above azlactone was reduced and hydrolyzed with hydriodic acid (d. 1.7), red phosphorus, and acetic anhydride to give 9 g. of 3-fluoro-dl-phenylalanine, the properties of which corresponded with those reported in the literature.

III. The Synthesis of 3,5-Diiodo-dl-Tyrosine.

As a comparison with the fluorinated amino-acids, 3,5-diiodo-dl-tyrosine was prepared. Since the method of Lamb and Robson[20], tried twice, gave very poor yields and a good deal of tar, a modification was used.

Fifty grams of 4-methoxybenzal phenyloxazolone-(5), prepared in the usual manner, was suspended in 500 ml. of hot 96% ethanol, and 15 g. of sodium hydroxide in 500 ml. of hot water was added. The mixture was stirred and boiled for fifteen minutes. On neutralization to congo red paper with dilute sulphuric acid, a yield of 47 g. of α-benzoylamino-p-methoxy cinnamic acid, m.p. 228°, was obtained. Catalytic reduction of the above compound

gave a 95% yield of α-benzoylamino-p-methoxy phenylalanine, of m.p. 173°. On refluxing the above compound with a mixture of 50% hydrobromic acid-50% acetic acid for twelve hours, an 80% yield of dl-tyrosine was obtained.

3,5-Diiodo-dl-tyrosine.--- To 8.6 g. of dl-tyrosine in 200 ml. of 8 N ammonium hydroxide solution was added gradually a 5% excess over the theoretical amount of potassium triiodide solution. After standing for one hour, the mixture was distilled until the smell of ammonia could no longer be detected, and the resulting precipitate was filtered off and crystallized first from 50% aqueous acetic acid, and later from boiling water. The yield was 7 g. of diiodo-dl-tyrosine, of m.p. 194°.

IV. The Synthesis of 3,5-Difluoro-dl-Tyrosine.

Considerable difficulty was experienced in the preparation of the difluoro compounds necessary as starting compounds for the above amino acid. Several unsuccessful attempts were made; but although these did not succeed in producing the desired products, they served to increase greatly our knowledge of conditions of fluorination. For instance, some compounds gave soluble fluoborates, others, as nitro-compounds, decomposed explosively when heated; and attempts to prepare a difluoro compound from a diamine by diazotization of both groups at once resulted in very poor yields if any. Consequently, these unsuccessful methods are described below along with those which were finally used to obtain the desired compounds.

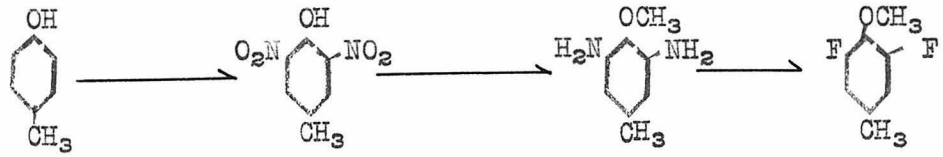
A. From M-Phenylene Diamine.

This reaction was attempted more to find out if such difluorinations could be practicable than to produce a starting material for the preparation of difluorotyrosine.

To a solution of 15 g. of sodium nitrite in 50 ml. of water and 80 ml. of concd. hydrochloric acid was added with stirring at 0°, 18 g. of m-phenylene diamine hydrochloride. To the resulting clear solution was added 85 ml. of 40% fluoboric acid, prepared as described above. The resulting precipitate was filtered, washed with alcohol and ether, and dried in vacuo over potassium hydroxide. The yield was 21 g. of the diazonium fluoborate.

On decomposition, however, the salt gave only a few drops of reddish oil.

B. From Para-Cresol.



This scheme was first attempted by Schiemann[21], who reported indefinite results. It was therefore decided to attempt it again before discarding it as a possibility.

2,6-Dinitro-p-Cresol.[22] To an ice-cooled 3-neck flask equipped with a stirrer and two dropping funnels, were added simultaneously a solution of

90 g. of p-cresol and 90 ml. of acetic acid and a solution of 135 g. of fuming nitric acid in 135 ml. of acetic acid. When both the solutions had been added, the reaction mixture was stirred another hour and then poured into ice-water. The precipitate was filtered off and recrystallized from ethanol to give 100 g. of the desired dinitro compound, of m.p. 78-79°.

Sodium 2,6-Dinitro-p-Cresolate.---- The above prepared phenol was dissolved in an excess of boiling sodium carbonate solution. On cooling, the solution deposited red needles of the sodium salt, which was collected, giving 100 g. of the desired compound.

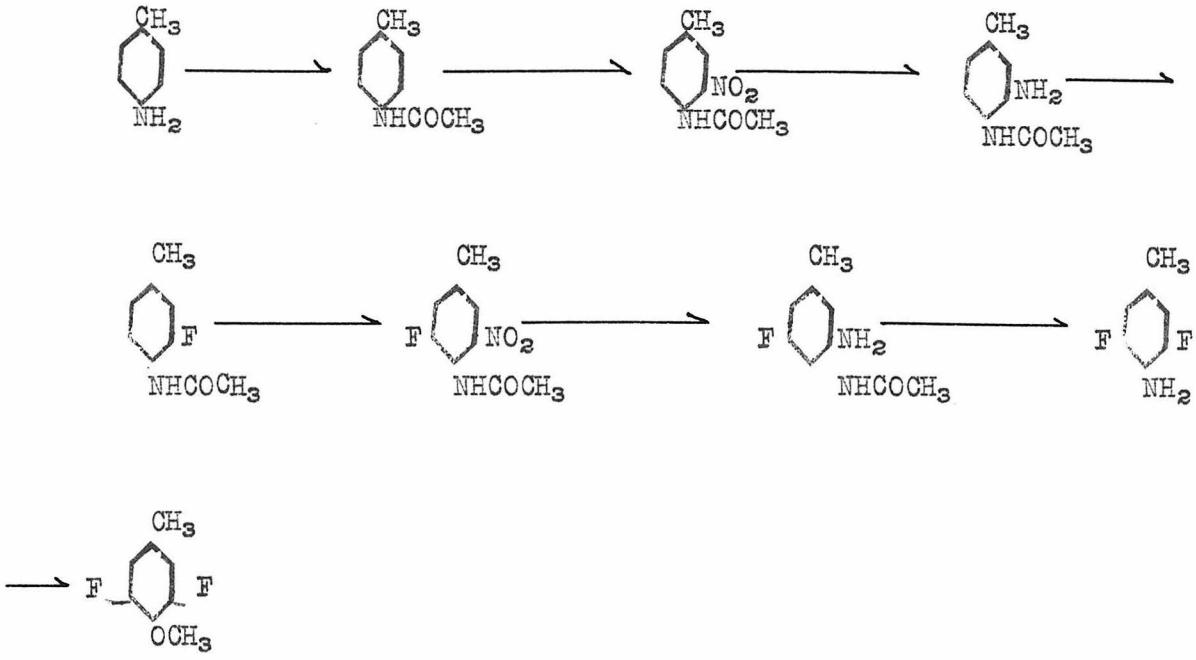
2,6-Dinitro-p-Cresol Methyl Ether.[23]---- The above sodium salt was heated during three hours at 120-140° with 80 ml. of toluene and 205 ml. of methyl sulfate, after which the mixture was left overnight to crystallize. The precipitate was filtered, washed with water, and recrystallized three times from alcohol. There was obtained 42 g. of the desired ether of m.p. 121-123°.

2,6-Diamino-p-Cresol Methyl Ether.---- Catalytic reduction of the above compound with platinum oxide and hydrogen in absolute ethanol gave a solution from which 36 g. of the diamine hydrochloride was obtained with dry hydrogen chloride.

2,6-Difluoro-p-Cresol Methyl Ether.---- To a cooled solution of 13 g. of sodium nitrite in 75 ml. of concd. hydrochloric acid and 45 ml. of water was added 18 g. of the above hydrochloride. To the resulting solution was added 80 ml. of fluoboric acid, prepared as described above. The precipitate

was filtered and washed with ethanol and ether to give only 8 g. of the diazonium fluoborate. Decomposition of this salt gave only a few drops of a red liquid, as had been reported by Schiemann.

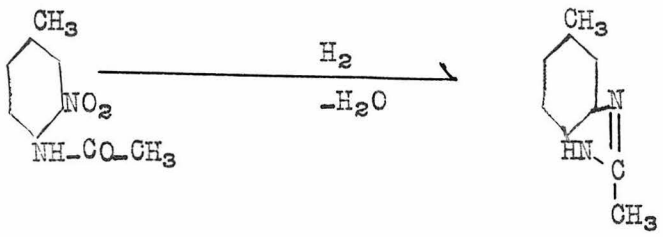
C. From p-Toluidine.



Although this series of reactions seemed a bit tedious, it was attempted because of the ease and directness of the preparations involved. The reason it failed is interesting since the reaction involved is generally used on paper without regard for the practical limitations. Several early workers[24] reported the formation of an insoluble compound when they attempted to reduce 2-nitro-p-acetotoluide in various ways. Bossneck[24a] found that reduction with iron in an acetic acid-alcohol solution gave the desired acetamino toluidine of m.p. 131°, but reported the formation of the

insoluble compound of m.p. 198°, when using other methods. He and Hobrecker[24b] gave the formula (below) for the compound formed; while he as well as Zincke and Lawson[24c] found that an attempt at diazotization gave another insoluble white compound. We found that catalytic hydrogenation with platinic oxide in ethanol gave the same compound of m.p. 197°; and, in view of the reported difficulty in diazotization, abandoned the process. In the reduction, the compound actually produced was found to be 2,5-dimethyl-benzimidazol; while in the diazotization, the product is 1-acetyl-5-methyl-benztriazol. These reactions, including the correct formula for the latter compound, are given below.

(1)



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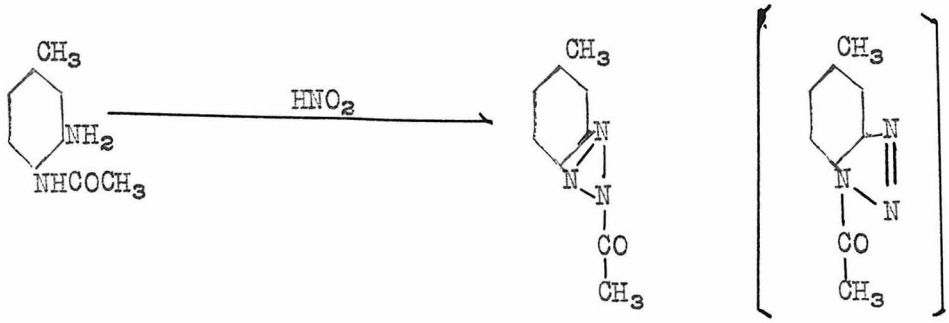
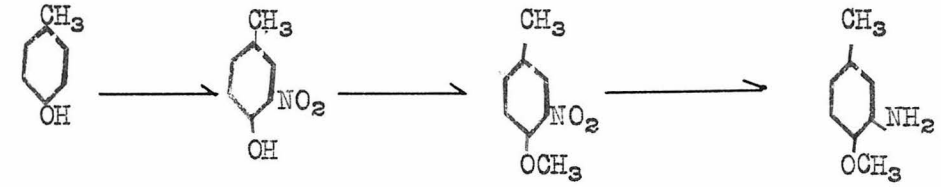


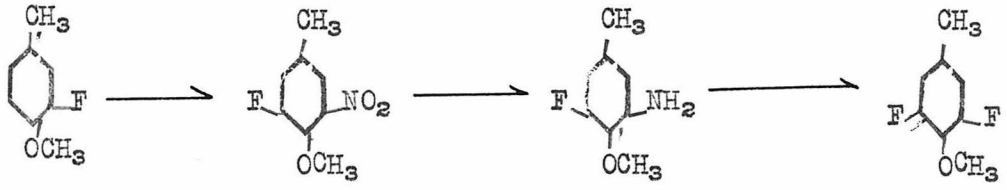
2-Nitro-p-Aceto-Toluide.---- To a solution of 400 g. of nitric acid (d. 1.45) stirred at 30-40°, was added 100 g. of p-aceto-toluide (prepared in the usual manner from p-toluidine). After all the toluide had been added, the solution was stirred another hour and poured into ice-water. The precipitate

was recovered by filtration and was recrystallized from alcohol. There was obtained 100 g. of 2-nitro-p-aceto-toluide of m.p. 93-94°.

Reduction of this compound with platinic oxide in ethanol gave, not the expected amine of m.p. 131°, but a white solid of m.p. 197°.

D. From Mononitro-p-Cresol.





This method, while it did not give the desired product, still served as a check on later experiments, since the fluoro methoxy toluene obtained could be oxidized to a fluoroanisic acid identical with that obtained later by oxidation of fluoroacetophenone (see below). Nitration of this same compound gave, significantly, not the expected nitro derivative, in which the nitro-group is in the ortho position to the methoxy group, but that in which it is ortho to the methyl and para to the fluorine. In other words, it was found that the combined directive influences of fluorine and methyl out-weighed that of methoxy alone.

Mononitro-p-Cresol.--- This starting material was prepared in two ways, one of which proved to be greatly superior to the other.

a) From p-toluidine[25].--- To a stirred suspension of 428 g. of p-toluidine in 392 g. of concd. sulfuric acid and 2200 ml. of ice-water was added 252 g. of nitric acid (d. 1.5) in an equal volume of water. To the resulting suspension was added gradually a solution of 280 g. of sodium nitrite in 500 ml. of water. The clear diazonium solution thus obtained was carefully decomposed in the following manner. Into a five-liter flask heated by a water-bath and equipped with an efficient reflux condenser was introduced 200 ml. of the diazonium solution. This solution immediately began to decompose; and when the reaction had subsided somewhat, the remainder of the solution was added slowly through a separatory funnel. On completion of the reaction, the resulting mixture was extracted with ether, the ether solution was washed with water, dried somewhat over sodium sulfate, and freed of solvent. The resulting oil was distilled giving only 170 g. of the desired product, distilling at 125° (25 mm.), and a large amount of unnitrated p-cresol.

b) By Nitration of p-Cresol[26].--- To a stirred solution of 100 g. of p-cresol dissolved in 200 ml. of benzene was added, at 0° (not 20° as in the article), a solution of 150 g. of nitric acid (d. 1.45) in 150 ml. of water. After the addition, the solution was stirred another hour and then steam distilled until the benzene had been removed. The receiver

was then changed, and the steam distillation was continued till the o-nitro-p-cresol had been collected. The yield was 90% (126 g.) as reported in the literature.

3-Nitro-4-Methoxy-Toluene[27].--- To a solution of 100 g. of the above nitrocresol in a solution of 32 g. of potassium hydroxide in 600 ml. of water was added, with stirring at 70°, 100 g. of methyl sulfate. The solution was stirred for two hours at 70° and was then cooled and extracted with ether. On evaporation of the dried ether solution there was obtained an oil, which distilled at 131° (at 4 mm.) to give 80 g. of the desired 3-nitro-4-methoxy-toluene.

3-Amino-4-Methoxy-Toluene.--- The above nitro compound was reduced catalytically with platinic oxide in ethanol. The hydrochloride of the amine was precipitated from the ethanol solution with dry hydrogen chloride.

3-Fluoro-4-Methoxy-Toluene.--- To a solution of 35 g. of the above amine in 70 ml. of concd. hydrochloric acid solution was added with stirring at 0° a solution of 22 g. of sodium nitrite in 50 ml. of water. To the resulting clear solution was added 80 ml. of 40% fluoboric acid solution. The resulting precipitate was filtered, washed with ethanol and ether and dried *in vacuo* over potassium hydroxide.

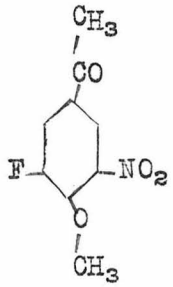
This salt decomposed smoothly in the usual manner to give 11 g. of the desired fluoro-methoxy-toluene.

3-Fluoro-4-Methoxy-6-Nitro-Toluene.--- Since the nitration in acetic acid apparently gave no results, the following procedure was adopted.

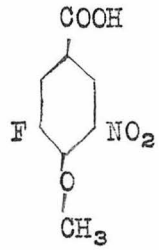
To a solution of 4.2 g. of the starting material in 10 ml. of concd. sulfuric acid at 0° was added with stirring 2 g. of nitric acid (d. 1.5) in 2 ml. of concd. sulfuric acid. The resulting solution was poured into ice-water, and the precipitate was collected by filtration and recrystallized from ethanol to give crystals of m.p. 107-108°.

In preliminary tests on small samples, the above nitro compound was found to be readily reduced catalytically with platinic oxide in ethanol; the resulting amine was found to diazotize smoothly to give an insoluble diazonium fluoborate.

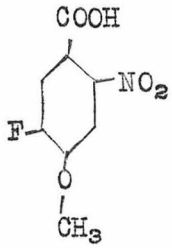
Oxidation with alkaline potassium permanganate of a small sample of the above fluoro methoxy nitrotoluene gave an acid of m.p. 194-195°. Later oxidation experiments on a compound of known structure (I) to give the acid (II) with a melting point of 166-167° proved that the previously prepared acid had the structure (III), and that the above nitration product of fluoro nitro methoxy toluene had the structure (IV).



(I)



(II)



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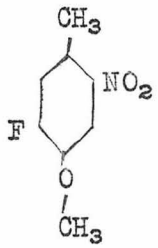
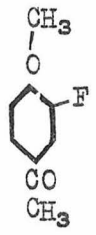


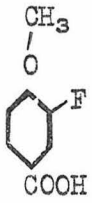
(IV)

Howevever, when a small sample of the original fluoro methoxy toluene was oxidized in the same manner with alkaline potassium permanganate, an acid was obtained which was identical (mixed m.p. of 208-210°) with that

obtained in the oxidation of the product obtained by acetylation of fluoroanisole. Therefore the structure of the product of acetylation was (V), and that of the acid produced by the oxidation was (VI).



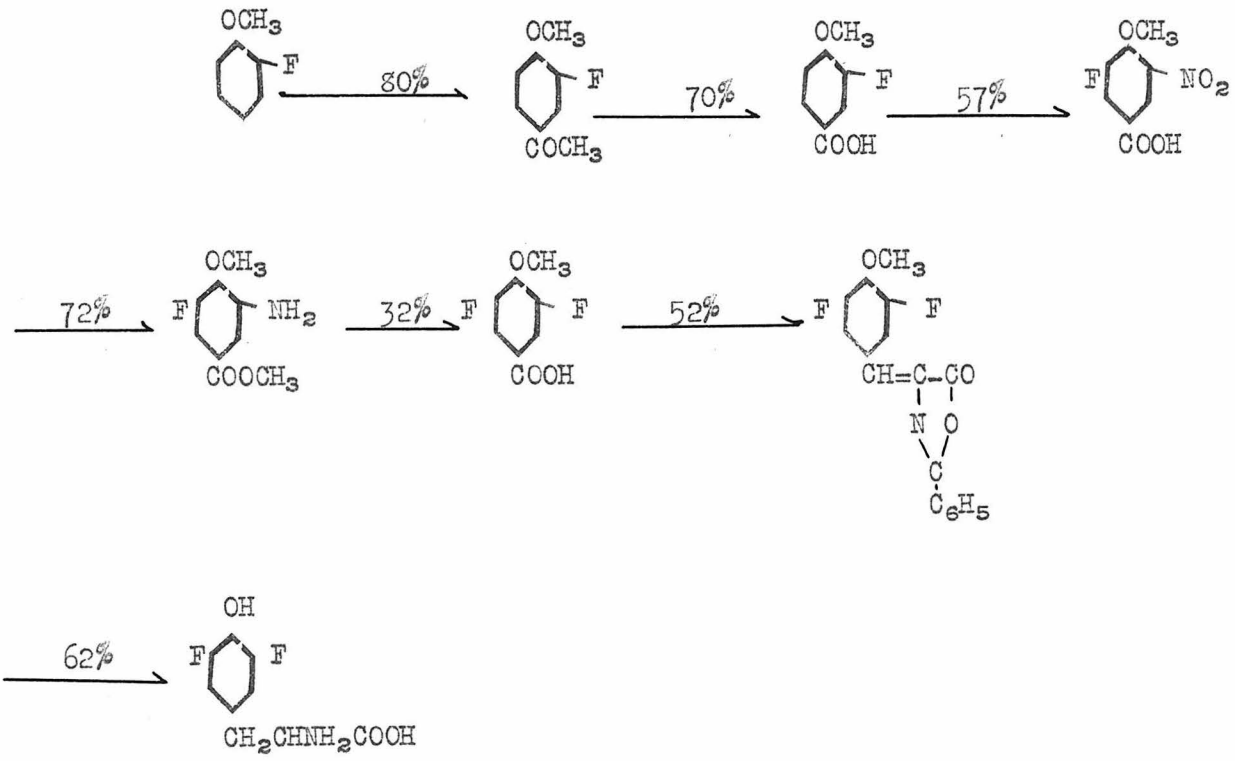
(V)



(VI)

This acetylation reaction, therefore, afforded a convenient method for preparing difluoro compounds; and in the first publication on the subject[9], this was the method employed.

E. From o-Fluoro-Anisole.



3-Fluoro-4-Methoxy-Acetophenone.--- To 750 g. of o-fluoro-anisole in 2.4 ℓ. of carbon disulphide was added, with cooling, 1.80 kg. of aluminum chloride and 6.5 g. of acetic anhydride[28]. The reaction mixture was refluxed for one hour and was then poured into ice-water. The solid was recovered by filtration and recrystallized from ethanol with the aid of charcoal. The yield of 3-fluoro-4-methoxyacetophenone, m.p. 92°, was 700-800 g. or 70-80% of the theoretical amount.

Anal. Calcd. for $C_9H_9O_2F$ (168): C, 64.3; H, 5.4. Found: C, 64.1; H, 5.3.

3-Fluoroanisic Acid[13].--- Two hundred and seventy-five grams of potassium permanganate dissolved in the minimum quantity of hot water was added to 100 g. of 3-fluoro-4-methoxyacetophenone suspended in 1 ℓ. of water (at 80°) containing 2 g. of potassium hydroxide. After the reaction was completed the manganese dioxide was removed by filtration, and the filtrate was acidified with dilute sulfuric acid; the solution was then heated to 95-98°, and sufficient ethanol was added to dissolve the precipitate. On cooling, 70.3 g. (70%) of 3-fluoroanisic acid, m.p. 208-210°, crystallized from the solution.

Anal. Calcd. for $C_8H_7O_3F$ (170.0): C, 56.5; H, 4.1. Found: C, 56.6; H, 4.2.

3-Fluoro-5-Nitroanisic Acid.--- Forty-eight grams of 3-fluoroanisic acid was added in small portions to 250 ml. of nitric acid (d. 1.5), with efficient stirring at -5°. The clear solution was allowed to stand at 0°

for two hours and was then poured into ice-water. The 3-fluoro-5-nitroanisic acid separated as a white solid which was recovered by filtration, washed with cold water, and recrystallized from benzene, m.p. 166°. The yield was 35 g. or 57% of the theoretical amount.

Anal. Calcd. for $C_8H_7O_5NF$ (215.0): C, 44.7; H, 2.8; N, 6.5. Found: C, 44.7; H, 2.9; N, 6.6.

Methyl 3-Fluoro-4-Methoxy-5-Nitrobenzoate.--- The esterification of 3-fluoro-5-nitroanisic acid was conducted in the usual manner with methanol and hydrogen chloride. From 200 g. of the acid 160 g. (80%) of the ester, b.p. 128-131° (3 mm.) was obtained.

Anal. Calcd. for $C_9H_8O_5NF$ (229.0): N, 6.1. Found: N, 6.4.

Methyl 3-Fluoro-4-Methoxy-5-Aminobenzoate.--- One hundred and forty grams of the above nitro compound was dissolved in methanol and reduced catalytically with the aid of platinic oxide and hydrogen at 40 pounds. Upon evaporation of the solvent, 110 g. (90%) of the amine of m.p. 53° was obtained. After recrystallization from isopropyl ether, the compound melted at 55°.

Anal. Calcd. for $C_9H_{10}O_3NF$ (199.0): N, 7.0; Found: N, 7.2.

3,5-Difluoroanisic Acid.--- To 30 g. of methyl 3-fluoro-4-methoxy-5-aminobenzoate in 25 ml. of concd. hydrochloric acid was added, at -5°, 11 g. of sodium nitrite dissolved in the minimum quantity of water. 60 ml. of fluoboric acid, prepared as usual, was then added to the clear solution of

the diazotized amine, still maintaining the temperature at -5°. After the reaction mixture had stood for some time at 0°, the insoluble diazonium fluoborate was recovered by filtration, washed in the usual manner, and dried *in vacuo* over solid potassium hydroxide. The yield was 38 g. or 89% of the theoretical amount. The diazonium fluoborate (38 g.) was decomposed by dry distillation, and 13 g. of methyl 3,5-difluoro-4-methoxy benzoate was obtained. Without further purification the ester was saponified with alcoholic potassium hydroxide, and upon acidification of the reaction mixture, 9.0 g. (32%) of crude 3,5-difluoroanisic acid was obtained. Several recrystallizations from benzene gave 8.0 g. (28%) of pure difluoroanisic acid of m.p. 162°.

Anal. Calcd. for $C_8H_6O_3F_2$ (188.0): C, 51.1; H, 3.2. Found: C, 51.3; H, 3.3.

4-(3',5'-Difluoro-4'-methoxybenzal)-2-phenyloxazolone-5.--- Eight grams of 3,5-difluoroanisic acid was heated on a steam-bath for three hours with thionyl chloride. The excess reagent was removed by distillation *in vacuo* at 100°, and the crude acid chloride, m.p. 15-20°, was converted without further purification into the desired 3,5-difluoroanisaldehyde. The reduction was accomplished by refluxing a solution of the acid chloride in 20 ml. of xylene, containing 1 g. of 5% palladium barium sulfate catalyst and 45mg. of quinoline sulfur poison[29], while passing in hydrogen for three hours. At the end of this time no more hydrogen chloride was being evolved, and the reaction mixture was filtered, extracted with a small

quantity of dilute hydrochloric acid, washed with water, dried over sodium sulfate, and the xylene was removed in vacuo. The residual liquid aldehyde was converted into the azlactone in the usual manner, and from this reaction 7.0 g. (52%) of 4-(3',5'-difluoro-4'-methoxy)2-phenyl oxazolone-5, of m.p. 165-169° (dec.) was obtained.

Anal. Calcd. for $C_{17}H_{11}O_3NF_2$ (315): N, 4.4. Found: N, 4.6.

α-N-Benzoylamino-3,5-Difluoro-4-Methoxycinnamic Acid.---- The above azlactone was saponified with alcoholic sodium hydroxide, and, after acidification of the reaction mixture with dilute sulfuric acid, α-N-benzoylamino-3,5-difluoro-4-methoxycinnamic acid, m.p. 200-201°, was obtained.

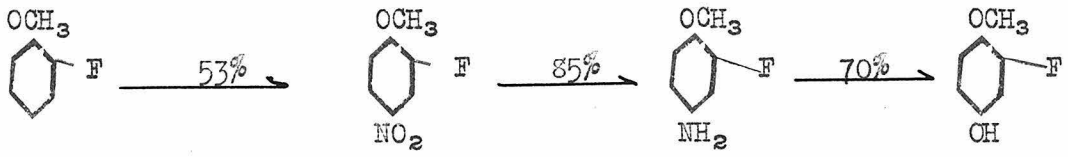
Anal. Calcd. for $C_{17}H_{13}O_4NF_2$ (333): C, 61.2; H, 3.9; N, 4.2. Found: C, 61.0; H, 4.0; N, 4.1.

3,5-Difluoro-dl-Tyrosine.---- Seven grams of the above prepared 4-(3',5'-difluoro-4'-methoxybenzal)-2-phenyloxazolone-5, and 5 g. of red phosphorus were refluxed for five hours with a solution of 65 ml. of hydriodic acid (d. 1.7) and 50 ml. of acetic anhydride. The reaction mixture was then worked up in the usual manner, and 3.7 g. of the crude amino-acid was obtained. Recrystallization from water gave 3.0 g. (62%) of 3,5-difluoro-dl-tyrosine, of m.p. 263-265° (dec.).

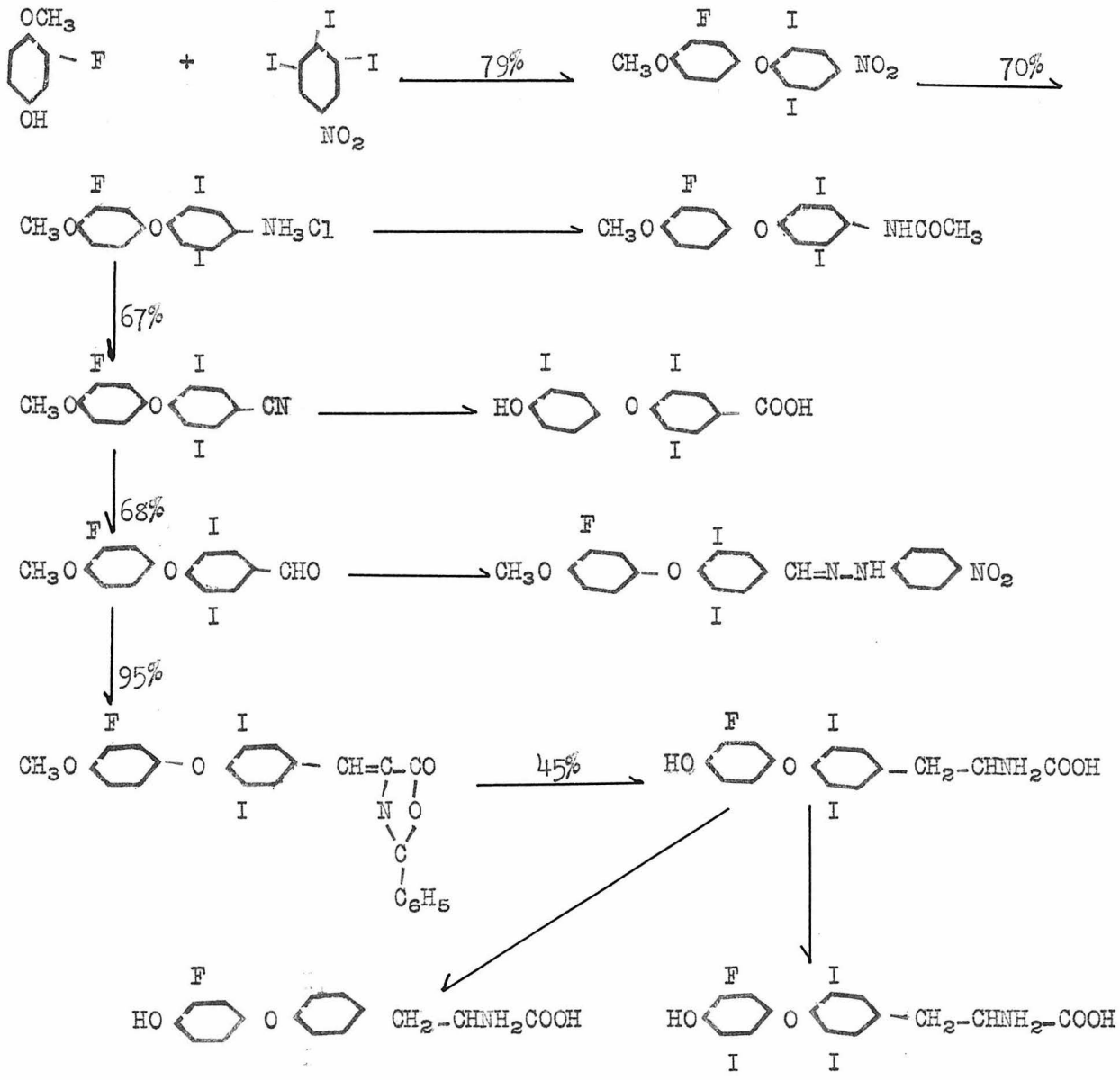
Anal. Calcd. for $C_9H_9O_3NF_2$ (217): C, 49.8; H, 4.2; N, 6.5. Found: C, 49.7; H, 4.3; N, 6.3.

V. The Synthesis of 3'-Fluoro-dl-Thyronine and Some of Its Iodinated Derivatives.

The starting material for the synthesis of the above thyroxine analogues was 3-fluoro-4-methoxy phenol, which was prepared by the following series of reactions:



From this point the general procedure followed by Harington and Barger[30] in the synthesis of thyroxine was used, as is shown in the following series of reactions:


79%
70%
67%
68%
95%
45%

2-Fluoro-4-Nitroanisole[9,31].--- To a vigorously stirred solution of 250 g. of o-fluoroanisole[31] in 825 ml. of acetic anhydride was added, over a period of four hours, a solution of 87 ml. of nitric acid (d. 1.5) in 40 ml. of acetic acid, the temperature being maintained at -10°. The reaction mixture was stirred an additional two hours, while the temperature was allowed to rise to 25°, and was poured into a large excess of ice-water, and left overnight in order to complete the hydrolysis of the acetic anhydride. The crystalline precipitate was recovered by filtration and recrystallized from a 1:1 mixture of methanol and ethanol to give 130 g. of 2-fluoro-4-nitroanisole of m.p. 101-103°. Upon recrystallization from the above solvent or from isopropyl ether, the m.p. was raised to 104.0 to 104.5°, in agreement with the literature.

The residual oil obtained as a by-product in the above nitration was cooled to -30°, and an additional 20 g. of pure nitrofluoroanisole was recovered, thereby raising the yield of the latter compound to 45% of the theoretical amount. The 180 g. of oil recovered from the above operation was reduced catalytically with platinic oxide in ethanol at 40 pounds of hydrogen. The solvent was evaporated, and the reduction product was taken up in ether and extracted with dilute hydrochloric acid. On evaporation of the washed, dried ether solution, 40 g. of o-fluoroanisole was obtained. The total yield of 2-fluoro-4-nitroanisole, based on starting material used was thus 53%.

On neutralization with sodium carbonate of the acid solution formed in the above reaction there was obtained a red oil, which was extracted

with ether. The ether solution was washed with dilute sodium carbonate solution and then with water until neutrality, and finally dried over anhydrous sodium sulfate. On evaporation of the ether there was obtained 12.7 g. of a light red oil, which was acetylated with acetic anhydride. An oil (11 g.) was thus obtained, which was distilled at 95-100° at 0.1 mm. to give 5.5 g. of a colorless crystalline solid of m.p. 39°, and a solid residue. Analysis of the distillate gave C, 58.9; H, 5.8, in agreement with the theory for an acetylated amino fluoroanisole (see below).

Part of the free amine was distilled at 76° (2 mm.) leaving a crystalline residue. Diazotization of the distilled amine proceeded smoothly, and an insoluble fluoborate was obtained in the usual manner. This decomposed nicely to give some liquid distillate.

This series of experiments was made to ascertain whether or not the nitration of fluoroanisole could be used as a start in the preparation of difluoroanisole, which would be useful in the preparation of later difluorothyronine derivatives. Since these latter compounds, however, were prepared by a simpler more definite series of reactions, the above reactions are merely interesting with regard for the amounts and types of isomers obtained in the nitration of fluoroanisole. Since it has now been found that Ingold[31c] did not have fluoroanisole, and so could not have obtained any evidence on the nitration of that compound, this problem is especially interesting. Experiments are now being conducted which may give a more definite answer to this problem.

2-Fluoro-4-Aminoanisole[9,31].---- This compound was obtained by the catalytic reduction, with platinic oxide in ethanol at 40 pounds pressure of hydrogen, of 2-fluoro-4-nitroanisole. The crude product was recrystallized from methanol, giving a yield of 85% of 2-fluoro-4-aminoanisole, of m.p. 83.0-83.5°.

Anal. Calcd. for C_7H_8ONF (141): C, 59.6; H, 5.7; N, 9.9. Found: C, 59.6; H, 5.7; N, 10.0.

The above amine was acetylated in the usual manner with acetic anhydride to give the N-acetyl compound of m.p. 113-114°.

Anal. Calcd. for $C_9H_{10}O_2NF$ (183.2): C, 59.0; H, 5.5; N, 7.7. Found: C, 59.0; H, 5.3; N, 7.7.

3-Fluoro-4-Methoxyphenol.---- Twenty-one grams of 2-fluoro-4-aminoanisole was suspended in a solution prepared by adding 20 ml. of concd. sulfuric acid to 60 g. of ice. After the addition of a second 60 g. portion of ice, the amine was diazotized by introducing 12 g. of sodium nitrite dissolved in the minimum quantity of water. The excess nitrous acid was removed with urea, and the solution, diluted to 500 ml., was dropped slowly into a distilling flask containing a boiling solution of 75 g. of anhydrous sodium sulfate in 100 g. of concd. sulfuric acid and 50 ml. of water. The solution in the flask was maintained at 130-135° during the addition of the diazonium solution by regulating the rate of addition of the latter solution. After all the diazonium solution had been added, 200 ml. of water was introduced into the flask in a similar manner, the temperature still being maintained at 130-135°. The phenol was recovered from the

distillate by extracting the latter with ether. The ether extract was dried, the solvent was removed, and the residual 15 g. of crude phenol (70%) was distilled at 90° (0.4 mm.). Upon recrystallization from a mixture of benzene and ligroin, 3-fluoro-4-methoxyphenol, m.p. 54-55°, was obtained.

Anal. Calcd. for $C_7H_7O_2F$ (142.1): C, 59.1; H, 4.9.
Found: C, 58.8; H, 5.0.

3,5-Diiodo-4-(3'-Fluoro-4'-Methoxyphenoxy)-Nitrobenzene[30,31]. --- A mixture of 100 g. of triiodonitrobenzene[32], 41 g. (a 1.4 molar proportion) of 3-fluoro-4-methoxyphenol, 60 g. of freshly dehydrated anhydrous potassium carbonate, and 275 ml. of freshly distilled 2-pentanone was refluxed in an oil-bath for six hours. Water was added to dissolve the salts, and the 2-pentanone and other volatile products were removed by steam distillation. When 1.5 ℓ. of distillate had been collected, the mixture was allowed to cool, and the water was poured off the solid, but somewhat tarry, mass. The latter was treated with 100 ml. of methanol, which dissolved out the tar and caused the condensation product to become crystalline. The light brown solid was collected by filtration, washed with methanol, and recrystallized from 2-butanone, giving 80 g. (79%) of a light yellow product, of m.p. 126-127°. Upon repeated recrystallizations from the same solvent, the m.p. was raised to 127-129°.

Anal. Calcd. for $C_{13}H_8O_4NI_2F$ (515.0): C, 30.3; H, 1.6; N, 2.7.
Found: C, 30.4; H, 1.5; N, 2.9.

3,5-Diiodo-4-(3'-Fluoro-4'-Methoxyphenoxy)-Aniline Hydrochloride.--- To a hot solution of 75 g. of 3,5-diiodo-4-(3'-fluoro-4'-methoxyphenoxy)-nitrobenzene in 385 ml. of acetic acid was added, in small portions, 117 g. of powdered stannous chloride dihydrate. The reaction was conducted as previously described, and, upon passing dry hydrogen chloride into the ethereal solution of the amine, 53 g. (70%) of 3,5-diiodo-4-(3'-fluoro-4'-methoxyphenoxy)-aniline hydrochloride, m.p. 200° (after a preliminary sintering) was obtained.

Anal. Calcd. for $C_{13}H_{11}O_2NI_2FCl$ (521.5): C, 29.9; H, 2.1; N, 2.7. Found: C, 29.9; H, 2.3; N, 3.0.

3,5-Diiodo-4-(3'-Fluoro-4'-Methoxyphenoxy)-Acetanilide.--- The free base was liberated from the above hydrochloride by shaking an ethereal suspension of the latter with N aqueous sodium hydroxide. The base, recovered from the ethereal solution, was acetylated with acetic anhydride, and after several recrystallizations from ethanol, the acetamino compound was obtained as colorless platelets, m.p. 199-200°.

Anal. Calcd. for $C_{15}H_{12}NO_3I_2F$ (527.1): C, 34.2; H, 2.3; N, 2.7. Found: C, 34.2; H, 2.5; N, 2.6.

3,5-Diiodo-4-(3'-Fluoro-4'-Methoxyphenoxy)-Benzonitrile.--- To a well-stirred suspension of 41 g. of 3,5-diiodo-4-(3'-fluoro-4'-methoxyphenoxy)-aniline hydrochloride in 410 ml. of glacial acetic acid was added, at 15-20°, 8 g. of butyl nitrite. After stirring for an additional thirty minutes, the resulting clear solution was poured, with vigorous stirring, into a hot solution prepared by adding 235 g. of potassium cyanide in

410 ml. of water to 215 g. of copper sulfate pentahydrate in 820 ml. of water. The stirring was continued for one hour, and after one hour the precipitate was collected. The solid was dehydrated with the aid of benzene and then extracted with three 200 ml. portions of boiling benzene. Upon evaporation of the solvent a dark brown crystalline solid was obtained which was distilled at 0.1 mm. pressure from an oil-bath at 250°. The distillate was taken up in chloroform and washed with aqueous sodium bisulfite. Removal of the solvent gave 26 g. (67%) of a light yellow crystalline solid, which, after two recrystallizations from ethanol, melted at 115-117°.

Anal. Calcd. for $C_{14}H_8O_2NI_2F$ (495.0): C, 34.0; H, 1.6; N, 2.8. Found: C, 34.2; H, 1.9; N, 2.6.

3,5-Diiodo-4-(3'-Fluoro-4'-Hydroxyphenoxy)-Benzoic Acid.--- A specimen of 3,5-diiodo-4-(3'-fluoro-4'-methoxyphenoxy)-benzonitrile was hydrolyzed with a 1:1 mixture of acetic acid and hydriodic acid (d. 1.7) for thirty minutes. The hydrolysate was diluted with cold water, the precipitate was recovered, extracted with dilute ammonium hydroxide; the extract was filtered, and the filtrate was acidified with 6 N hydrochloric acid. The precipitated acid was collected and recrystallized from a 50% aqueous ethanol solution giving a white crystalline compound of m.p. 237-238°.

Anal. Calcd. for $C_{13}H_7O_4I_2F$ (500.0): C, 31.2; H, 1.4. Found: C, 31.4; H, 1.7.

3,5-Diiodo-4-(3'-Fluoro-4'-Methoxyphenoxy)-Benzaldehyde.--- Sixty grams of anhydrous stannous chloride was suspended in 300 ml. of anhydrous ether, and dry hydrogen chloride was passed into the suspension, at 0°, until all of the solid had dissolved. To this solution was added a solution of 25 g. of 3,5-diiodo-4-(3'-fluoro-4'methoxyphenoxy)-benzonitrile in 175 ml. of dry chloroform. Hydrogen chloride was passed into the reaction mixture for an additional two hours, during which time a heavy yellow liquid separated. Upon standing overnight exposed to the atmosphere through a calcium chloride tube, the liquid was transformed into the solid stannic chloride double salt of the aldimine hydrochloride*. This was collected and hydrolyzed by boiling with 6 N hydrochloric acid. The aldimine double salt hydrolyzed rapidly, leaving a yellow glassy solid, which was crystallized from 70% aqueous acetic acid, giving 17 g. (68%) of 3,5-diiodo-4-(3'-fluoro-4-methoxyphenoxy)-benzaldehyde, m.p. 106-108°.

Anal. Calcd. for $C_{14}H_9O_3I_2F$ (498.0): C, 33.8; H, 1.8; Found: C, 33.8; H, 1.9.

The p-nitrophenylhydrazone of the above aldehyde was prepared by adding 0.2 g. of the aldehyde to an equivalent amount of p-nitrophenylhydrazine dissolved in hot glacial acetic acid. Upon recrystallization from glacial acetic acid, the hydrazone formed clusters of microscopic

*When the reaction was conducted in a pressure bottle very poor yields of aldehyde were obtained.

needles, m.p. 263-264°.

Anal. Calcd. for $C_{20}H_{14}O_4N_3I_2F$ (633.2): C, 37.9; H, 2.2; N, 6.6. Found: C, 37.8; H, 2.6; N, 6.8.

4-(3',5'-Diiodo-4'-(3''-Fluoro-4''-Methoxyphenoxy)-Benzal-2-Phenyloxazolone-5. An intimate mixture of 16 g. of 3,5-diiodo-4-(3'-fluoro-4'-methoxyphenoxy)-benzaldehyde, 8 g. of hippuric acid, 16 g. of anhydrous sodium acetate, and 60 ml. of acetic anhydride was heated on a boiling water-bath for one hour. The reaction mixture was poured, with stirring, into about 500 ml. of ice-water and allowed to stand until the acetic anhydride had hydrolyzed. The yellow solid was collected, washed with water and dried in vacuo. The 20 g. of azlactone, m.p. 180-190°, thus obtained is satisfactory for subsequent operations.

α-Benzoylamino-β-(3,5-Diiodo-4-(3'-Fluoro-4'-Methoxyphenoxy)-Phenyl)-Acrylic Acid.---- A portion of the above azlactone was added to 100 parts of a boiling solution of 1% sodium hydroxide in 70% ethanol, and the reaction mixture was boiled for ten minutes before acidification with dilute hydrochloric acid. The precipitated acid was collected and recrystallized several times from ethanol to give the crystalline acid, needles, m.p. 238-240°.

Anal. Calcd for $C_{23}H_{16}O_5NI_2F$ (659.2): C, 41.9; H, 2.4; N, 2.1. Found: C, 41.8; H, 2.4; N, 2.4.

dl-α-Amino-β-(3,5-Diiodo-4-(3'-Fluoro-4'-Hydroxyphenoxy)-Phenyl)-Propionic Acid.---- A mixture of 30 ml. of acetic anhydride, 30 ml. of hydriodic acid, (d. 1.7), 3 g. of red phosphorus, and 5 g. of the above prepared azlactone was refluxed for three hours. The hot solution was filtered through a sintered glass filter, and the filtrate was evaporated to dryness in vacuo. The residue was dissolved in 40 ml. of 2 N hydrochloric acid, the solution decolorized with carbon, filtered, and the amino acid precipitated by neutralization with 15 N ammonium hydroxide. The average yield was 1.8 g. or 42% of the theoretical amount. The precipitated amino acid was dissolved in 70% aqueous ethanol with the aid of a small quantity of dilute aqueous sodium hydroxide; the solution was filtered, and the filtrate was rapidly acidified with dilute acetic acid[30,33] to give 3'-fluoro-3,5-diiodo-dl-thyronine, platelets of m.p. 248° (dec.). Prior to analysis the amino acid was again recrystallized from alcoholic sodium hydroxide as before.

Anal. Calcd. for $C_{15}H_{12}O_4NI_2F$ (543.1): C, 33.2; H, 2.2; N, 2.6; I, 46.8 Found: C, 33.3; H, 2.6; N, 2.5; I, 47.0.

dl-α-Amino-β-(4-(3'-Fluoro-4'-Hydroxyphenoxy)-Phenyl)-Propionic Acid.---- 3'-fluoro-3,5-diiodo-dl-thyronine (2 g.) dissolved in 150 ml. of N aqueous potassium hydroxide was reduced with hydrogen in the presence of palladized calcium carbonate following the procedure described by Harington[34]. After removing the catalyst, the solution was acidified with acetic acid, and

the precipitated amino acid was recovered by filtration. After several recrystallizations from alcoholic sodium hydroxide (see above), the amino acid, platelets, m.p. 238° (dec.), possessed the following composition.

Anal. Calcd. for $C_{15}H_{14}O_4NF$ (291.3): C, 62.0; H, 4.8; N, 4.8. Found: C, 62.1; H, 5.1; N, 4.8.

dl-α-Amino-β-(3,5-Diiodo-4-(3'-Fluoro-5'-Iodo-4'-Hydroxyphenoxy)-Phenyl)-Propionic Acid.--- The stoichiometrical quantity of 1 M potassium triiodide solution was added slowly to a well-cooled solution of 2 g. of 3'-fluoro-3,5-diiodo-dl-thyronine in 40 ml. of 7 N aqueous ammonium hydroxide. During the course of addition a precipitate appeared. After standing for one hour at 0°, the solution was diluted with 50 ml. of water, and sufficient sodium bisulfite was added to remove any excess iodine. The reaction mixture was made acid to litmus by the addition of dilute hydrochloric acid. The precipitated amino acid was recovered, washed with water and alcohol and recrystallized several times from alcoholic sodium hydroxide (see above). The final product, m.p. 201° (dec.), possessed the following composition.

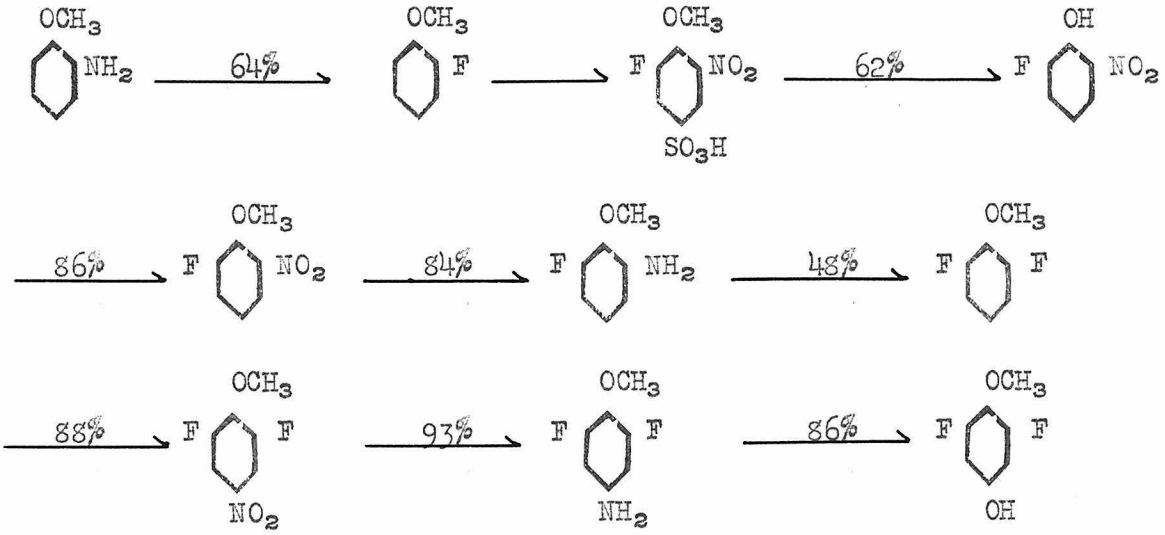
Anal. Calcd. for $C_{15}H_{11}O_4NI_3F$ (669.0): C, 26.9; H, 1.7; N, 2.1; I, 56.9. Found: C, 26.8; H, 2.1; N, 1.9; I, 58.0.

VI. The Synthesis of 3',5'-Difluoro-dl-Thyronine and 3,5-Diiodo-3',5'-Difluoro-dl Thyronine.

For the synthesis of difluoro thyronines, difluoro methoxy phenol was required. This could be prepared in low yield from difluoro anisic

acid amide (prepared before in the synthesis of difluoro tyrosine) by the Hoffmann degradation. However, a series of reactions was found which greatly decreased the number of steps and increased the yield.

The readily available fluroanisole was sulfonated and then nitrated in position 6. During the removal of the sulfonic acid group by distillation from strong acid, the compound was also demethylated. On methylation, reduction of the nitro group, and fluorination there was obtained difluoro anisole, which could be readily nitrated in the proper position. The steps in the preparation of difluoro methoxy phenol were as follows:



Experimental Part

Sodium 3-Fluoro-4-Methoxybenzenesulfonate.— Fluoroanisole was dissolved in five times its weight of concd. sulfuric acid. The solution became warm and turned light green, then became colorless and cooled. After standing at room temperature for six hours, the solution was poured onto

ice. The addition of sodium chloride to this solution gave a precipitate of the sodium salt, which was recrystallized from water for analysis.

Anal. Calcd. for $C_7H_6O_4FSNa$ (228.2): C, 36.8; H, 2.7; Na, 10.1. Found: C, 36.8; H, 2.9; Na, 10.1.

2,4-Dinitro-Fluoroanisole.--- To a cooled solution of fluoroanisole in five times its weight of concd. sulfuric acid as above was added, with cooling, a two molar portion of fuming nitric acid. After standing for one hour, the solution was poured onto ice, and the resulting oil was steam distilled. A light yellow oil was recovered with ether and was distilled, b.p. 100° at 0.5 mm.

Anal. Calcd. for $C_7H_5O_5N_2F$ (215.1): C, 38.9; H, 2.3; N, 13.0. Found: C, 39.2; H, 2.5; N, 13.2.

This reaction is peculiar since it indicates easy replacement of the sulfonic acid group by the nitro, whereas, as will be seen in a later reaction, this sulfonic acid group, if not replaced by the nitro group, is hydrolyzed only by high temperatures in strongly acid solutions.

Sodium 3-Fluoro-4-Methoxy-5-Nitrobenzene-Sulfonate.--- To a cooled solution of 6.0 g. of fluoro anisole in 25 ml. of concd. sulfuric acid was added 3.2 g. of nitric acid (d. 1.5). After standing for one hour, the solution was poured into ice-water, and a slight suspension was filtered off. On saturation of the resulting solution with salt, a white precipitate was obtained, which was recrystallized from water.

Anal. Calcd. for $C_7H_5O_6NFSNa$ (273.2): N, 5.1; Na, 8.4. Found: N, 5.2; Na, 8.4.

2-Fluoro-6-Nitrophenol.--- To a cooled solution of 25 g. of o-fluroanisole in 150 g. of concd. sulfuric acid was added with stirring 12.7 g. of nitric acid (d. 1.5). The solution was allowed to stand at room temperature for two hours and was then poured onto 150 g. of ice. A slight precipitate (found to be 4-nitro-fluroanisole) was removed by filtration, and 100 g. of solid potassium sulfate was added. The mixture, in a distilling flask, was heated on an oil bath while super-heated steam was passed in. At a temperature of 190°, a yellow oil, which solidified in the condenser, came over. This was collected and recrystallized from isopropyl ether to give 21 g. (62%) of yellow tablets and prisms of m.p. 92-94°. Analysis proved it to be the phenol.

Anal. Calcd. for $C_6H_4O_3NF$ (157.1): C, 45.9; H, 2.6; N, 8.9. Found: C, 45.9; H, 2.4; N, 8.9.

Reduction of a portion of the above fluoro-nitrophenol with hydrogen in the presence of platinic oxide gave the amine, which was recrystallized from methanol, m.p. 115°.

Anal. Calcd. for C_6H_6ONF (127.7): C, 56.8; H, 4.7. Found: C, 57.0; H, 4.6.

Since it was not certain at the time that the above compound was phenolic, a fluorination was tried. The amine could be diazotized, but the diazonium fluoroborate was apparently soluble and could not be

isolated. Methylation of a small sample of the nitro-fluorophenol with silver oxide and methyl iodide gave a liquid which was not investigated further.

At this point the problem was dropped, and the remainder of the work was carried out by A. A. Benson. However, it might be well to point out a few more of the synthetic possibilities of difluoroanisole.

In the preparation of difluoro tyrosine, a Gatterman aldehyde synthesis using difluoroanisole should give good yields of the required aldehyde. On the other hand, the sulfonic acid group could be removed by heating with molten potassium cyanide to give the nitrile, which could be reduced to the aldehyde or hydrolyzed to the amide or acid. In this manner a number of syntheses described in former papers might be shortened and made more efficient. Until some future date, when these experiments are actually tried, however, it is difficult to say just how important these improvements may be.

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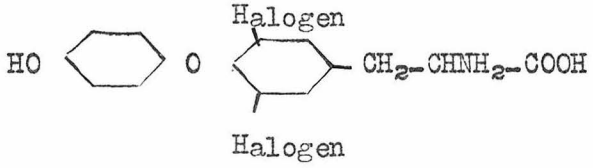
Part III

The Preparation of Isomers and Analogues of Thyroxine with Relation to a Proposed Hypothesis of the Relationship between Structure and Thyroxine-like Activity

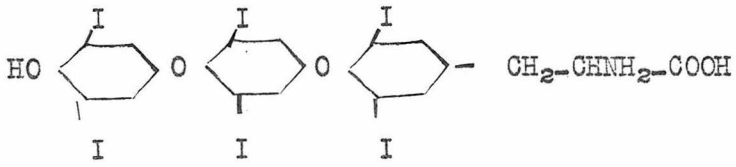
For a considerable time before the discovery of the chemical nature of thyroxine, it was believed that the thyroid gland is intimately involved in the processes of oxidation in the body. As early as 1917 it had been shown by Streuli and others that both the administration of thyroid material and thyroidectomy produce marked changes in the sensitiveness of the animal to lack of oxygen. Since then Deuel, Sandiford, Sandiford and Boothby[1] have shown that the consumption of oxygen and the output of carbon dioxide are primary results of thyroxine administration, and that the increased nitrogen elimination is probably of secondary importance. Thus it was proposed, even before Harington's synthesis of thyroxine, that whatever the chemical nature of the substance, it should be easily affected by oxidation-reduction processes, and in turn should be able to influence the velocity of oxidative processes occurring in the animal organism.

Since Harington's classical work[2] a good deal of research has been conducted on the oxidation-reduction reactions of the aromatic hydroxyl group. Adrenalin was examined by Kendall and Witzemann[3]; Pugh and Raper[4] have shown a relation between hydroxyl and quinone groups to the oxidation of amino acids in the presence of tyrosinase and oxygen; and, more recently, Kendall and others have investigated some of the oxidation-reduction properties of thyroxine itself. The results of these investigations led to the belief that an "active form" of thyroxine may be responsible for the sensitivity of the hormone to oxidizing agents.

Recently, considerable work has been done on the relationship of chemical structure to the physiological action in the thyroxine series. Harington[5] has found that the phenolic hydroxyl group, the diphenyl ether linkage, the iodine content, and the aminopropionic side chain are all necessary for activity. However, all the iodine is not necessary, since 3,5-diiodo-thyronine has been found to possess about one-fiftieth of the activity of the thyroxine, and the tetrabromo compound has been found active in some degree. Consequently, Harington set down the following structure as obligatory for the development of thyroxine-like activity:

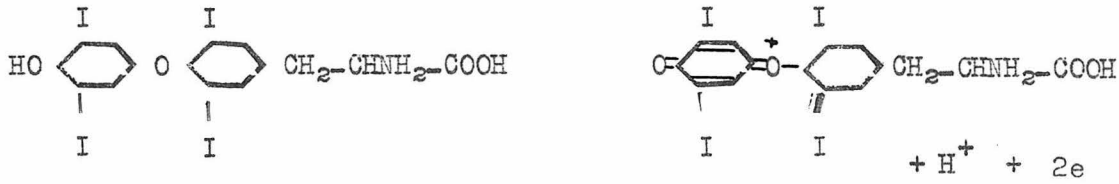


Bovarnick, Bloch and Foster[6] synthesized the following compound, which, according to Harington's postulate, might be expected to show some activity:



They reported it to be of no significant activity, but, as Niemann has since pointed out[7], since the authors did not adminster the compound at levels higher than approximately 6 mg./kg., and since a slight activity apparently was observed, it is not necessary to conclude, at least at present, that this compound is completely devoid of physiological activity.

Most recently Niemann proposed as a provisional working hypothesis that thyroxine-like activity, at least in the case of thyroxine itself, is dependent upon the establishment of the equilibrium:



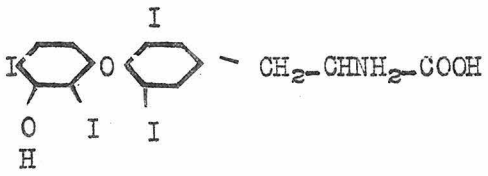
and predicted that those structures which do not permit the formation of a quinoid form will be inactive, and that the quantitative differences in the activity of those compounds which can form such structures are due to the influence of nuclear substituents on the oxidation-reduction potential of the systems as a whole. To test this hypothesis several experiments have been devised. Niemann and Redemann[7], synthesized an isomer of thyroxine in which the hydroxyl group in the second ring was shifted from position 4', as in thyroxine, to position 3', thus preventing the formation of a quinoid form. This compound, dl-3,5-diiodo-4-(2'-4'-diiodo-3'-hydroxyphenoxy)-phenylalanine (I), when tested on rats, was found to be inactive even in doses of 500 mg. per kg. of body weight.

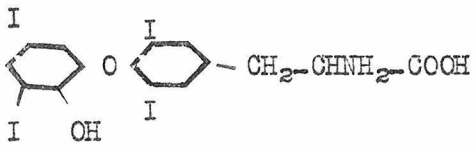
As a further test for this hypothesis, Niemann and Mead[8] synthesized the isomer dl-3,5-diiodo-4-(3',5'-diiodo-2'-hydroxyphenoxy)-phenylalanine (II), in which an ortho-quinone form is possible. This isomer, in accordance with the prediction, is physiologically active, the activity being about one-twenty-fifth of that of thyroxine*. The activity of both of

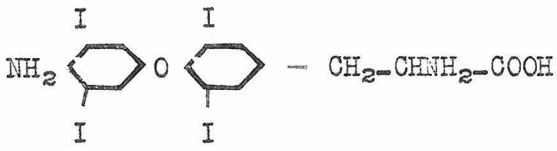
*We are indebted to Professor P. Phillips, Department of Biochemistry, University of Wisconsin, for the physiological work on these compounds.

these compounds, therefore, has supported the proposed hypothesis relating chemical structure to thyroxine-like activity.

As a continuation of these studies, the synthesis of an analogue of thyroxine containing an amino group in place of the hydroxyl has been attempted. In this compound, dl-3,5-diiodo-4-(3',5'-diiodo-4'-aminophenoxy)-phenylalanine (III), a quinone form is possible, and some activity might be predicted. This synthesis has not as yet been completed because of technical difficulties.

I. 

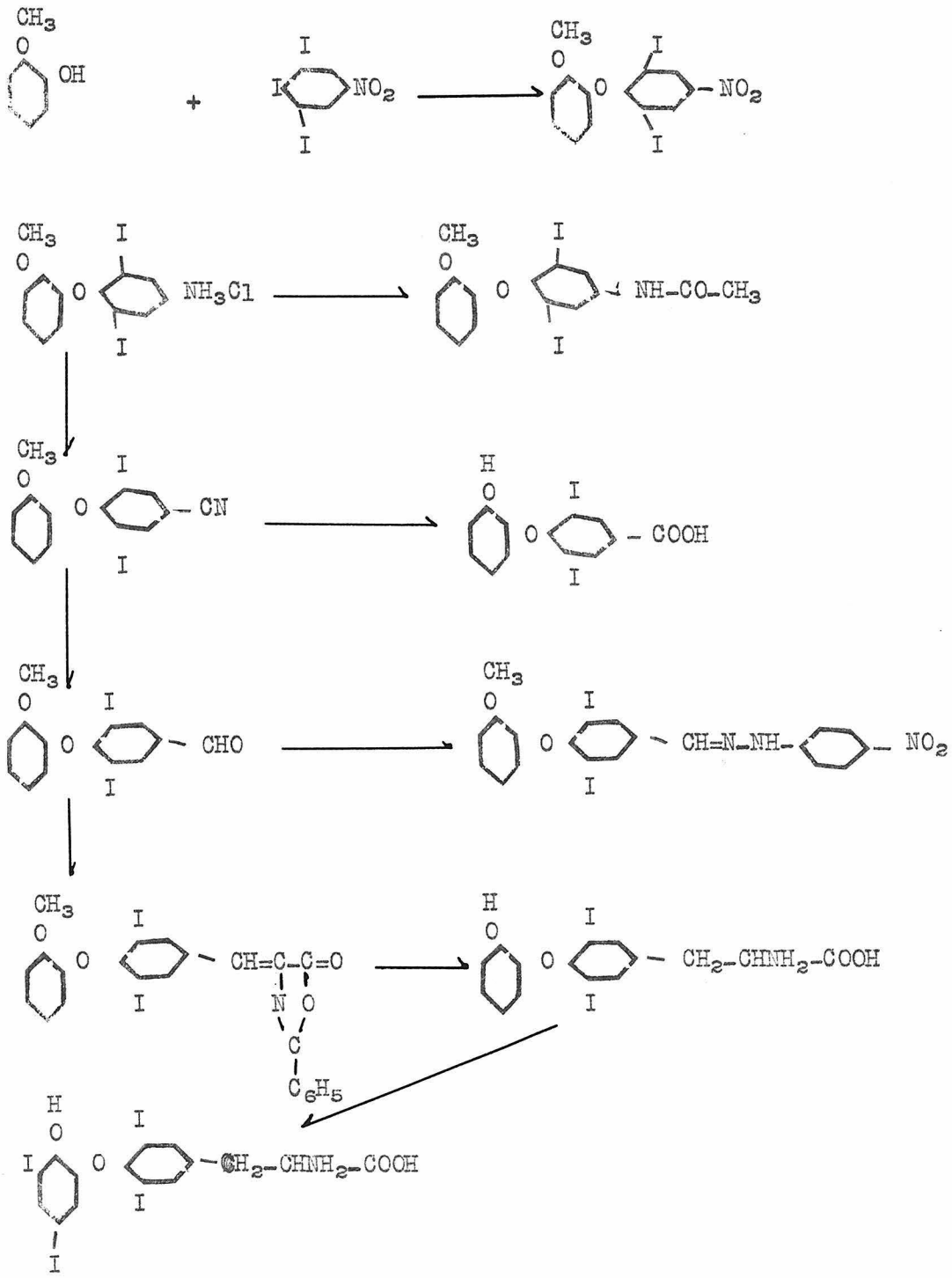
II. 

III. 

Experimental Part

I. The Synthesis of dl-Diiodo-4-(3'-5'-Diiodo-2'-Hydroxyphenoxy)-Phenylalanine.

The synthesis of this compound proceeded according to the following scheme:



3,5-Diiodo-4-(2'-Methoxyphenoxy)-Nitrobenzene.--- A mixture of 250 g. of triiodonitrobenzene[7], 90 g. of guaiacol, 155 g. of freshly dehydrated anhydrous potassium carbonate and 650 ml. of freshly distilled 2-pentanone was refluxed for six hours. Water was added, and the 2-pentanone and excess guaiacol were removed by steam distillation. The water was decanted, and, after cooling, the tarry mass was treated with about 500 ml. of methanol, which dissolved out most of the tar and caused the residue to solidify. 250 grams of this residue was recrystallized three times from 2-butanone to give 80 g. of light yellow crystals, m.p. 148-150°.

Anal. Calcd. for $C_{13}H_9O_4NI_2$ (497.0): C, 31.4; H, 1.8: N, 2.8. Found: C, 31.2; H, 2.0; N, 2.9.

This yield apparently could not be improved although the reaction was tried a great many times under various conditions. The impurity, which was a low-melting tarry solid, could not be identified or avoided. If a lower boiling solvent, as 2-butanone, was used, or if the time of refluxing was shortened, the product was found to contain some unreacted triiodonitrobenzene from which it could not be separated. This was true, also, if the potassium carbonate was dried in a porcelain instead of a nickel crucible. If, on the other hand, the reaction time was lengthened, more of the low-melting compound was formed; and when a little copper bronze was added as a catalyst, none of the desired product could be recovered from the resulting tar. Although the nature of the by-product could not be ascertained, the failure of the reaction is thought to be due to steric hindrance caused by the interaction of the ortho methoxy group and

the iodines of the first ring. In general, it was found that all the reactions of this compound were more difficult and gave lower yields than the corresponding reactions of other isomers or analogues of thyroxine.

3,5-Diiodo-4-(2'-Methoxyphenoxy)-Aniline Hydrochloride.---- To a hot solution of 75 g. of the above nitro-compound in 375 ml. of acetic acid was added, in small portions, 115 g. of powdered stannous chloride dihydrate; and the stannic chloride double salt of the amine was isolated as previously described. The stannic chloride double salt was ground in a mortar with 150 ml. of warm 50% sodium hydroxide solution; the suspension was diluted with 100 ml. of ice-water and exhaustively extracted with ether, in which the amine is not very soluble. Dry hydrogen chloride was passed into the dried ethereal extract precipitating the amine hydrochloride, which was then collected with the aid of acetone. The product (60 g.) melted at 237° after preliminary sintering.

Anal. Calcd. for $C_{13}H_{12}NI_2Cl$ (503.5): C, 31.0; H, 2.5; N, 3.0. Found: C, 30.9; H, 2.5; N, 3.0.

The free base was liberated from the above hydrochloride by shaking an ethereal suspension of the latter compound with 2 N aqueous sodium hydroxide. The base, recovered from the ethereal solution, was acetylated with acetic anhydride; and after several recrystallizations from ethanol, 3,5-diiodo-4-(2'-methoxyphenoxy)-acetanilide, m.p. 225-227°, was obtained as colorless platelets.

Anal. Calcd. for $C_{15}H_{13}NO_3I_2$ (509.1): C, 35.4; H, 2.6; N, 2.8. Found: C, 35.7; H, 2.6; N, 2.9.

3,5-Diiodo-4-(2'-Methoxyphenoxy)-Benzonitrile.--- Ten grams of butyl nitrite was added to a well-stirred suspension of 40 g. of the above amine hydrochloride in 400 ml. of acetic acid containing 5% of water. Upon warming to 50°, a clear orange solution was obtained, which was added, with stirring, to a solution prepared by adding 245 g. of potassium cyanide in 400 ml. of water to 215 g. of cupric sulfate pentahydrate in 800 ml. of water. The mixture was warmed to 80°, cooled and filtered; and the solid was first dehydrated and then extracted with hot benzene. The dark brown solution was decolorized by passing it through a column of activated alumina, and from the filtrate 31 g. of light yellow crystals was obtained. This product was then distilled at 0.2 mm. (bath temp. 225°) to give 24 g. of nitrile. The distillation residues were dissolved in benzene, passed through a column of activated alumina, freed from solvent, and again distilled to give a further 1.5 g. of nitrile, making a total of 25.5 g. The distilled nitrile was recrystallized from ethanol to give light yellow prisms of m.p. 135-137°.

Anal. Calcd. for $C_{14}H_9NO_4I_2$ (477.1): C, 35.3; H, 1.9; N, 2.9. Found: C, 35.2; H, 2.0; N, 2.8.

3,5-Diiodo-4-(2'-Methoxyphenoxy)-Benzaldehyde.--- Fourteen and one-half grams of the above prepared nitrile was treated with anhydrous stannous chloride as previously described to give 8 g. (55%) of the aldehyde,

m.p. 137-140°, after recrystallization from acetic acid. The p-nitrophenylhydrazone was prepared in the usual manner to give yellow needles of m.p. 249-250°.

Anal. Calcd. for $C_{23}H_{15}O_4NI_2$ (623.2): C, 44.3; H, 2.4; N, 2.3. Found: C, 44.5; H, 2.6; N, 2.1.

4-(3',5'-Diiodo-4'-(2"-Methoxyphenoxy)-Benzal)-2-Phenyloxazalone-5.---- A nearly quantitative yield of crude azlactone was obtained from the above aldehyde by following the procedure previously described. The crude azlactone was recrystallized from cellosolve to give yellow needles of m.p. 198-200°.

Anal. Calcd. for $C_{23}H_{15}O_4NI_2$ (623.5): C, 44.3; H, 2.4; N, 2.3. Found: C, 44.5; H, 2.6; N, 2.1.

dl-α-Amino-β-(3,5-Diiodo-4-(2'Hydroxyphenoxy)-Phenyl)-Propionic Acid.---- A mixture of 20 ml. of acetic anhydride, 20 ml. of hydriodic acid (d. 1.7), 3 g. of red phosphorus and 3 g. of the above azlactone was refluxed for four hours. The hot solution was filtered, and the filtrate was evaporated to dryness *in vacuo*. The residue was boiled for one minute with 2 N hydrochloric acid, cooled and filtered. The filtrate was heated just to boiling, exactly neutralized with dilute aqueous ammonia and immediately filtered. This filtrate was allowed to stand overnight at room temperature. The resulting precipitate was collected, washed with water and ethanol and again dissolved in 2 N hydrochloric acid and reprecipitated as described above. The crude amino acid thus obtained was then dissolved in

hot 80% ethanol with the aid of dilute aqueous sodium hydroxide; the solution was filtered, and the filtrate was adjusted to pH 6.0 with dilute acetic acid. After standing for some time, the amino acid crystallyzed as clusters of colorless needles, m.p. 240°, with decomposition. Approximately 0.3 g. of recrystallized amino acid was obtained from 3 g. of the azlactone.

Anal. Calcd. for $C_{15}H_{13}O_4NI_2$ (525.1): C, 34.3; H, 2.5; N, 2.7; I, 48.3. Found: C, 34.5; H, 2.8; N, 2.5; I, 48.6.

The product which made up the remainder of the yield was a low-melting solid soluble in dilute aqueous alkali but not in acids. It gave a test for phosphorus, but could not be hydrolyzed to give any identifiable product. The nature of this compound is not known, but it is thought that it may be a phosphoric amide.

dl-α-Amino-β-(3,5-Diiodo-4-(3',5'-Diiodo-2'-Hydroxyphenoxy)-Phenyl)-Propionic Acid.--- Iodine (0.28 g.) dissolved in 1 M potassium iodide solution was added, dropwise, to a chilled solution containing 0.277 g. of the above prepared amino acid in 10 ml. of 7 N ammonium hydroxide, and the reaction mixture was allowed to stand at 0° for one-half hour. After the addition of a small amount of sodium bisulfite solution to the reaction mixture, it was adjusted to pH 4 with dilute hydrochloric acid. The solid that had precipitated was collected and washed with water and ethanol. The crude amino acid was then recrystallized by dissolving it in 80% ethanol containing the requisite quantity of sodium hydroxide and

suddenly acidifying the solution with dilute acetic acid. The green basic solution became pink on acidification and deposited 0.33 g. of pale pink clusters of needles, m.p. 218-219°, with decomposition.

Anal. Calcd. for $C_{15}H_{11}O_4NI_2$ (776.9): C, 23.2; H, 1.4; N, 1.8; I, 65.3. Found: C, 23.5; H, 1.7; N, 1.9; I. 65.4.

II. The Synthesis of dl-Diiodo-4-(3',5'-Diiodo-4'-Aminophenoxy)-Phenylalanine.

p-Hydroxy-Acetanilide.--- Forty-three grams of p-nitrophenol in a solution of 100 ml. of glacial acetic acid and 50 ml. of acetic anhydride was reduced with hydrogen in the presence of platinic oxide. The resulting solution was poured into a liter of water, and the solution was evaporated until crystals began to separate. After cooling the crystals were filtered and then recrystallized from 2-butanone to give 38 g. of p-hydroxy acetanilide of m.p. 163-165°, as reported in the literature.

3,5-Diiodo-4-(4'-Acetylaminophenoxy)-Nitrobenzene.--- A mixture of 100 g. of triiodonitrobenzene, 42 g. of p-hydroxy acetanilide, 60 g. of freshly dehydrated anhydrous potassium carbonate and 250 ml. of freshly distilled 2-pentanone was refluxed in an oil-bath. During the first half hour a precipitate came down, but the heating was continued for four hours. The solid was then filtered, washed with water, dried thoroughly and recrystallized from cellosolve. Eighty grams of crystalline material of m.p. 247-247.5°, was obtained.

Anal. Calcd. for $C_{14}H_{10}O_4N_2I_2$ (524.0): C, 32.1; H, 1.9; N, 5.4. Found: C, 32.2; H, 2.1; N, 5.1.

3,5-Diiodo-4-(4'-Acetylaminophenoxy)-Aniline Hydrochloride.--- Several unsuccessful attempts to reduce the above nitro-compound were made before a procedure was finally found which gave good results.

To a hot solution of 35 g. of the above nitro-compound in 200 ml. of glacial acetic acid was added, in small portions, 52.5 g. of powdered stannous chloride dihydrate. When dry hydrogen chloride was passed into the solution, an oil precipitated. On the completion of this reaction, the mixture was cooled, five volumes of ether were added, and the solution was left overnight. The oil turned to a solid and became semi-crystalline. The ether was poured off, and acetone was added to the solid, which became crystalline and was filtered to give about 30 g. of the amine stannichloride double salt.

This salt was rubbed in a mortar with 40% sodium hydroxide solution; the resulting mixture was exhaustively extracted with ether. The ethereal solution was dried over sodium sulfate and treated with dry hydrogen chloride to give an oil. On treatment of this oil with acetone, a solid was obtained which was filtered to give 25 g. (88%) of the amine hydrochloride of m.p. 200° (after preliminary sintering).

Anal. Calcd. for $C_{14}H_{13}N_2O_2I_2Cl$ (530.6): C, 31.7; H, 2.5; N, 5.3. Found: C, 31.6; H, 3.0; N, 5.0.

From the above ether solution some of the amine was recovered and recrystallized from toluene to give needles of m.p. 187-191°. Treatment

of this amine with acetic anhydride gave 3,5-diiodo-4-(4'-acetylamino-phenoxy)-acetanilide, m.p. 313-315°.

Anal. Calcd. for $C_{16}H_{14}O_3N_2I_2$ (536.1): C, 35.8; H, 2.6; N, 5.2. Found: C, 36.3; H, 3.0; N, 5.2.

When this amine was treated with butyl nitrite in glacial acetic acid solution, an orange precipitate was formed. This substance could not be made to react further, and could not be dissolved in any reasonable excess of acetic acid.

Further experiments are being made in an effort to carry out this diaotization.

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Part IV

A Carotenoid from Bovine Spinal Cord

It is well known that the carotenoids, which are so widely distributed in plants, also play an important rôle in the animal kingdon, where, because of their solubility in fats, they belong to the class of lipochromes.

Although vertebrates are apparently unable to synthesize these polyene pigments, they are able to store the ingested carotenoids in almost every organ of the body. Careful investigations have shown that they occur in many organs such as the blood, liver, depot fats and retina, and also in milk, butter, egg yolks, etc.

The functions of these animal carotenoids, although not thoroughly investigated, are known in part. They are used as pigments in the bodies of many animals, as in the feathers of some birds; while their rôle as precursors of vitamin A is of utmost importance to the animal.

The vertebrates, although they cannot synthesize the lipochromes, show some selectivity in the types of pigments which each animal will store. Many mammals, as the horse and cow, deposit only carotenes (polyene hydrocarbons); most birds store only the zanthophylls (polyene alcohols). Some animals such as the pig, do not deposit lipochromes in the fat, having colorless fats; while others, as man and frog, are able to accumulate both types of carotenoids.

Since Professor Carl Niemann was investigating the lipids of bovine spinal cord, it seemed a good opportunity to investigate the carotenoids in this material. The starting material for the experiments described below was an alcoholic extract of fresh spinal cord. The carotenoid fraction was separated in the usual manner. The quantity was extremely

small so that only a trace of β-carotene could be identified, after a chromatographic treatment.

Experimental Part

The alcoholic extract was made by allowing 23 kg. of fresh cord to stand for nine days in 64 ℓ. of 96% ethanol. The mixture was filtered through cheese-cloth, and the dark yellow filtrate was worked up in portions.

Forty liters of this solution was concentrated at reduced pressure in an atmosphere of nitrogen, until the volume had reached 8 ℓ. The resulting solution was kept at 5° overnight and was filtered from precipitated sterols, which were recrystallized from ethanol and were saved for another experiment (not yet completed).

To the filtrate 1 ℓ. of ligroin (b.p. 60-70°) was added, and the solution was continuously washed with water during one day. Large amounts of a white solid substance (probably lecithins and cephalins) were brought down in the aqueous phase by this treatment. These were put aside for further experiment. When further washing did not precipitate any more solids, ether was added, the water was drained off, and the pale yellow solution was allowed to stand overnight over a layer of concd. methanolic potassium hydroxide. The liquid was washed until it was alkali free and was dried over sodium sulfate. It was next evaporated to dryness, and the residue was taken up in 25 ml. of ligroin.

This solution was chromatographed on activated alumina giving one

main yellow zone and two very faint zones. The former was eluted with alcohol and transferred to ligroin. The extinction maxima of this fraction corresponded to that of β-carotene (520 and 484 mμ), while the amount, as estimated in a photometer, corresponded to about 0.02 mg. in about 15 kg. This pigment was epiphasic in the partition test.

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Part V

Studies on the Structure of Sphingosine

Although sphingosine occurs in almost every organ of the animal body, it was not until 1880 that Thudichum[1], the "father of brain chemistry", isolated it as the sulfate by hydrolysis of phrenosin. Phrenosin, which had been found earlier by Thudichum by appropriate treatment of protagon (the so called white matter), is a nitrogen-containing phosphorus-free substance. It was found, by hydrolysis, to be composed of one molecule of sphingosine, one of galactose, and one of cerebronic acid, which was thought to be a twenty-four carbon α-hydroxy fatty acid, but which has since been found by Chibnall and Piper[2] to be a mixture of α-hydroxy acids from eighteen to twenty-six carbon atoms.

In this same class of compounds, termed cerebrosides, are found other types of compounds, namely kerasin, nervon, and oxynervon, differing from phrenosin only in the fatty acid. Kerasin contains lignoceric acid, found by Chibnall and Piper[2] to be a mixture of straight chain fatty acids of twenty to twenty-eight carbon atoms; nervon contains nervonic acid, a mixture of unsaturated straight chain acids; while oxynervon is thought to contain α-hydroxy monounsaturated acids.

Closely related to the cerebrosides are the sphyngomylins, which, on hydrolysis, give sphingosine, a fatty acid of one of the types mentioned above, choline, and phosphoric acid.

Although the function of both of these types of compounds in the organism is not known, they are known to occur together with other types of fats throughout the animal body, especially in the nervous tissue,

in which they are found in large quantities. Together with the neutral fats, sterol esters and other phosphatides (phosphorus-containing compounds as sphingomylin) these types of compounds make up the class of lipids, or fats.

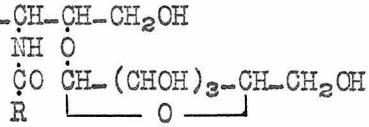
Sphingosine itself, from the analysis of $C_{17}H_{35}O_2N$ reported by Thudichum and others[3,4], was thought to be an amino dihydroxy heptadecene, until the analyses of Klenk[5,6] showed that the formula must contain eighteen carbon atoms. That it contains a double bond was shown first by Levene and Jacobs[7] by hydrogenation, and later by the oxidative degradation of the compound with chromic oxide, which gave myristic acid[5], proving that the double bond is in the 4 position.

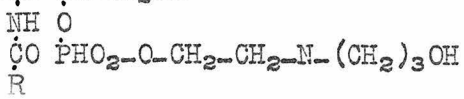
The nature of the substituent groups was shown by the fact that sphingosine could form a triacetate which contained no amino nitrogen (van Slyke). Also, the ozone splitting of this triacetate gave myristic acid and a dihydroxyaminobutyric acid, which had a specific rotation of -34°. Klenk[6], who carried out this work, reduced the dihydroxyamino acid with hydriodic acid and phosphorus to an optically inactive aminobutyric acid of m.p. 280-285°. Since this agreed more or less with the literature for α-amino-n-butyric acid (285°); and because the β and γ amino-n-butyric acids had lower melting points (185° and 202° respectively), Klenk thought that his amino acid must be 2-amino-3,4-dihydroxybutyric acid, giving (I) as the formula for sphingosine.

Because the original cerebroside did not show any free amino nitrogen (van Slyke), it is known that the amino group occurs as an

amide of the particular fatty acid. It was also shown that the galactose residue is joined to one oxygen, but that the other must be free, since methylation of dihydropsychosin (galactosido-sphingosine) followed by splitting with dilute sulfuric acid gave the monomethoxy dihydrosphingosine[8]. Thus the formula of a cerebroside was thought to be (II), while that of sphingomylin was thought to be (III).

(I). $CH_3-(CH_2)_{12}-CH=CH-CHNH_2-CHOH-CH_2OH$

(II).


(III).


There has been some doubt, however, about the validity of Klenk's work, especially since he did not characterize his amino acid thoroughly. Recently Seydel, working under Ruzicka (thesis), prepared N-acetyl dihydrosphingosine and found that with lead tetraacetate and periodic acid very poor yields of formaldehyde were obtained; whereas 1:2-glycols are known to be split easily and quantitatively by these reagents. Seydel therefore proposed the following as a possible structure for sphingosine:

$$CH_3-(CH_2)_{12}-CH=CH-CHOH-CHNH_2-CH_2OH$$

He did not go any farther into his investigation of structure other

than to attempt the synthesis of some of the possibilities.

Our purpose, in undertaking this research, is to determine with certainty, first the location of the two hydroxyls and the amino group and their configuration, and next to determine the configuration about the double bond.

In order to accomplish this, it was necessary to obtain large amounts of reasonably pure sphingosine.

For this purpose we first prepared a large quantity of cerebroside-sphingomylin fraction using a method developed by Professor Niemann to cut down the loss involved in the more drastic methods employed before this. In this method, desiccated bovine spinal cord was allowed to stand for several days each with successive portions of 96% ethanol. It was then pulverized and extracted with boiling ethanol, the cerebroside-sphingomylin (or C-S) fraction precipitating from the ethanol solution on cooling.

Since hydrolysis of the C-S fraction with methanolic hydrochloric acid followed by precipitation of the sphingosine as the sulfate is a long and tedious process; and since, at first, it was not possible to obtain good yields of pure sphingosine by hydrolysis of the sulfate, we decided to attempt the direct purification of the ether solution of crude sphingosine by chromatographic adsorption.

This procedure was possible since it had been found* that sphingosine itself exhibits a blue-green fluorescence under the quartz

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*Seymore Bernstein, Calif. Inst., 1940.

lamp, making it possible to detect the base on the Tswett column. While this method gave some very interesting results, it failed because of an unforeseen phenomenon. It was found, only after an extensive investigation was nearing completion, that impure sphingosine apparently undergoes a far-reaching decomposition when it is merely allowed to stand in ether or benzene solution. This was first apparent from the fact that solutions of sphingosine in either of these solvents, after standing a week, began to smell noticeably of ammonia. In addition to this, it was found that the products actually isolated on the column gave, when acetylated, not the expected triacetyl sphingosine, but compounds which analyzed as high molecular weight ethers or esters of sphingosine, of the general formula $C_{38}H_{71}NO_5$.

This decomposition not only made it impossible to obtain any pure sphingosine by this method, but generally hindered the chromatographic experiments, since each fraction, although obtained as a single zone in one column, would again form several zones when rechromatographed.

The chromatographic technique, however, as a means of obtaining the pure base, is not entirely without results. In a later experiment using freshly prepared sphingosine, and performing the operation as soon as solution in the benzene had been accomplished*, a large very faintly fluorescent zone was obtained. After elution of this zone with ethanol there was obtained a substance which on crystallization from ether, gave an apparently pure crystalline sample of sphingosine.

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*Even here it was noticed that after five minutes the solution had changed from colorless to light yellow.

Another interesting result of the chromatographic experiments has been the separation of pure sterols from the crude hydrolysis product, and the separation of an oil containing, among other substances, the liquid methyl esters of various fatty acids. These fractions will be investigated later.

In a continuation of the main problem, several different techniques were employed.

Sphingosine sulfate was isolated both from the above-mentioned C-S fraction and from cerebroside purified after the manner of Rosenheim[9,10] with pyridine, or of Page[11] with tetralin.

During the hydrolysis, the operations were carried out as quickly as possible so as not to leave the free sphingosine in alkaline or neutral solution any longer than was necessary. A white crystalline analytically pure sphingosine sulfate was obtained. This was hydrolyzed merely by shaking it with 10% aqueous sodium hydroxide solution instead of by heating it with alcoholic sodium hydroxide. This gave a product of much better appearance.

From here, the search has taken two main directions. One has been the degradation of triacetyl sphingosine to the amino-acid obtained by Klenk, and the subsequent investigation and characterization of this compound. In order to do this it is necessary to obtain the dihydroxy triacetyl spingosine by hydroxylation of the double bond. Perbenzoic acid, silver iodobenzoate, potassium permanganate, and hydrogen peroxide were tried without success. Even bromine would

not add to the double bond, the starting material being obtained in pure form in each case. It is thought that the samples used, although they had the correct melting point and other physical constants, must either have been prepared from naturally occurring dihydrosphingosine or may have isomerized about the double bond to give a more resistant isomer of sphingosine. A similar fact has apparently been noticed by Klenk[6] in the ozonolysis of triacetyl spingosine; while in the case of the sulfate, a type of isomerization has already been reported by Niemann[12]. In any case, attempts are now being made to carry out these reactions on other samples of triacetyl sphingosine.

The other attempt to correlate the structure of sphingosine with that of a known compound consists of the preparation of hydroxy octadecanes both from sphingosine and from known compounds. To eliminate the amino group from spingosine we are taking advantage of a reaction reported by von Braun[13]. Dihydrosphingosine was prepared and benzoylated. This tribenzoyl dihydrosphingosine was melted with phosphorus pentachloride to produce the dibenzoylhydroxychlorooctadecane, which will be reduced and hydrolyzed to the glycol. The reactions are as follows:

$$C_{13}H_{27}-CH=CH-CHOH-CHNH_2-CH_2OH \longrightarrow C_{15}H_{31}-CHOH-CHNH_2-CH_2OH$$

$$\longrightarrow C_{15}H_{31}-\underset{\underset{\underset{Ph}{CO}}{O}}{CH}-\underset{\underset{\underset{Ph}{CO}}{NH}}{CH}-\underset{\underset{\underset{Ph}{CO}}{O}}{CH_2} \longrightarrow C_{15}H_{31}-\underset{\underset{\underset{C_6H_5}{CO}}{O}}{CH}-\underset{Cl}{CH}-\underset{\underset{\underset{C_6H_5}{CO}}{O}}{CH_2}$$

$$\longrightarrow C_{15}H_{31}-CHOH-CH_2-CH_2OH$$

This reaction had been carried to the chloride, and synthesis of the 1:3-glycol is also under way.

Experimental Part

Preparation of C-S Fraction.--- Twenty-three kg. of desiccated bovine spinal cord was allowed to stand in 64 ℓ. of 96% ethanol at room temperature for nine days. The ethanol was decanted through cheese-cloth, and 54 ℓ. of ethanol was added to the residue. This suspension was allowed to stand for fifteen days, and the ethanol was again decanted. The extraction was repeated once more, and the residue was dried in air at room temperature*.

The dried tissue was powdered in a Wiley mill and also in a ball mill until all could pass through a 60-mesh screen. This material was extracted with alcohol in small portions in the following manner.

To 2.3 ℓ. of boiling 96% ethanol was added 350 g. of the pulverized tissue. The mixture was heated on the boiling water-bath for five minutes and was then filtered. The filtrate was immediately chilled in an ice-salt bath, and the residue was introduced into 1.6 ℓ. of boiling ethanol and again extracted as described above. The residue from this extraction was subjected to three additional extractions using in each instance 1.6 ℓ. of ethanol. The residue remaining after the final extraction was dried in air at 25° and was put aside for later experiment.

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*This work was done by Dr. D. S. Breslow, Calif. Inst., (1941).

The ethanol extracts were kept at 5° for one week, and the precipitates that had formed were collected on a suction filter, washed with cold ethanol and dried *in vacuo* over sulfuric acid. The solids so obtained were designated C-S fractions.

From 17.4 kg. of tissue treated in this manner there was obtained 5.5 kg. of C-S fraction. This represents 24% of the dry tissue or 8.1% of the fresh cord. There was also obtained 6.2 kg. of substance insoluble in the hot ethanol, representing 27.1% of dry tissue or 9.0% of the fresh cord. Evaporation of the alcoholic solutions after removal of the C-S fraction gave a mass of crystals. This was also put aside for further experiment.

Hydrolysis of the C-S Fraction.---- One hundred gram portions of this fraction were refluxed forty-eight hours with one liter of 2 N methanolic hydrogen chloride. The alcoholysate was transferred to 250 ml. centrifuge bottles, and the latter were stored at 5° for three days. The chilled bottles and their contents were centrifuged, and the supernatant liquid was decanted from the solid esters, which were then washed with the minimum quantity of cold methanol. The combined supernatant liquid and washings were distilled until 250 ml. of residue remained in the flask. To this concentrate was added 200 ml. of 5 N sodium hydroxide and 1.5 ℓ. of water. The resulting mixture was transferred to a Koch type extraction apparatus and extracted with ether until the aqueous phase was exhausted. The ether phase was washed several times with half-saturated salt solution and was

finally dried over sodium sulfate. The dried ethereal extract was freed of solvent; the residue was taken up in 30 ml. of methanol, and 10% methanolic sulfuric acid was added until the solution was barely acid to litmus. The precipitate which had formed was allowed to stand overnight at room temperature and was then collected by filtration and dried in vacuo over sulfuric acid. The 138 g. of crude sphingosine sulfate thus obtained was recrystallized in 30 g. portions by dissolving each portion in 1300 ml. of boiling absolute ethanol, filtering the solution, and allowing the filtrate to stand at 25° for two days. The crystalline precipitate was collected and dried in vacuo over sulfuric acid. The yield was 45 g. of pure sphingosine sulfate.

The Preparation of Cerebroside from C-S Fraction[9,10].— Two hundred and twenty-three grams of the above prepared C-S fraction was extracted in a soxhlet type extractor with hexane for thirty-six hours. The insoluble portion was removed from the extractor and dried in vacuo over paraffin. The yield was 133 g. of waxy solid. This product was combined with that from a similar experiment, and the resulting 250 g. was dissolved in 750 ml. of pyridine at 50°. The solution was allowed to cool to room temperature, and the resulting waxy precipitate was centrifuged down. The supernatant solutions were combined, concentrated at reduced pressure to about 200 ml., and poured into 700 ml. of acetone. The resulting precipitate was filtered off

and dried *in vacuo* over sulfuric acid. The free-flowing white solid thus obtained weighed 125 g.

Hydrolysis of the Cerebroside.--- One hundred and twenty-five grams of the above prepared cerebroside was refluxed for forty-eight hours with 1.25 ℓ. of 4 N methanolic hydrogen chloride. The alcoholysate was cooled to 5° and the precipitated esters were removed by filtration. One-half liter of water was added to the clear filtrate, and the resulting solution was extracted with two successive portions of petroleum ether (b.p. 30-60°) to remove the remaining esters. The resulting aqueous alcoholic solution was reduced *in vacuo* to about one liter and was then treated with 10% aqueous sodium hydroxide until permanently alkaline. The basic solution was extracted in a separatory funnel with three 300 ml. portions of ether; and the combined ether solutions were washed free of alkali and dried over sodium sulfate. The dried ethereal extract was freed of solvent; the residue was taken up in 75 ml. of absolute ethanol, and 4% ethanolic sulfuric acid was added until the solution was just acid to litmus. The resulting precipitate was filtered and washed with acetone to give 18 g. of white solid sphingosine sulfate. Recrystallization of this sulfate in the usual manner gave 11 g. of pure product.

Anal. Calcd. for $C_{36}H_{76}N_2O_8S$ (697.0): C, 62.0; H, 11.0; N, 4.0. Found: C, 62.2; H, 10.9; N, 4.3.

Chromatographic Investigation of the Crude Hydrolysis Product*---

After extended investigations it was found that the most suitable adsorbent for the purpose was Mefford's technical zinc carbonate. This substance was freed from a trace of fluorescent organic material by treatment with benzene in a percolator. The resulting pure zinc carbonate was dried first in air at room temperature, and then at 80°. It was then sifted through a 60-mesh screen and was mixed with one-fifth its weight of cellite (diatomaceous earth). Throughout the following experiments this adsorbent was used.

The ether solution of the alcoholysis product of the C-S fraction (obtained as described above) was dried over sodium sulfate, freed from solvent and dissolved in benzene. This solution was chromatographed in 20 ml. portions (found to contain 4.27 g.) on a number 6 tube, and was washed with benzene until the filtrate was no longer fluorescent. At this point, the material adsorbed on the column had separated into several zones (Fig. I) which were easily visible under the ultra-violet lamp. Although the edges of these zones were rather indistinct, the column was, nevertheless, removed from the tube and divided into three parts; the first distinct band (see Fig. I), the second distinct band, and the top quarter of the non-fluorescent portion (found to contain all the non-fluorescent material). Each portion was eluted with hot ethanol, and the resulting solutions were

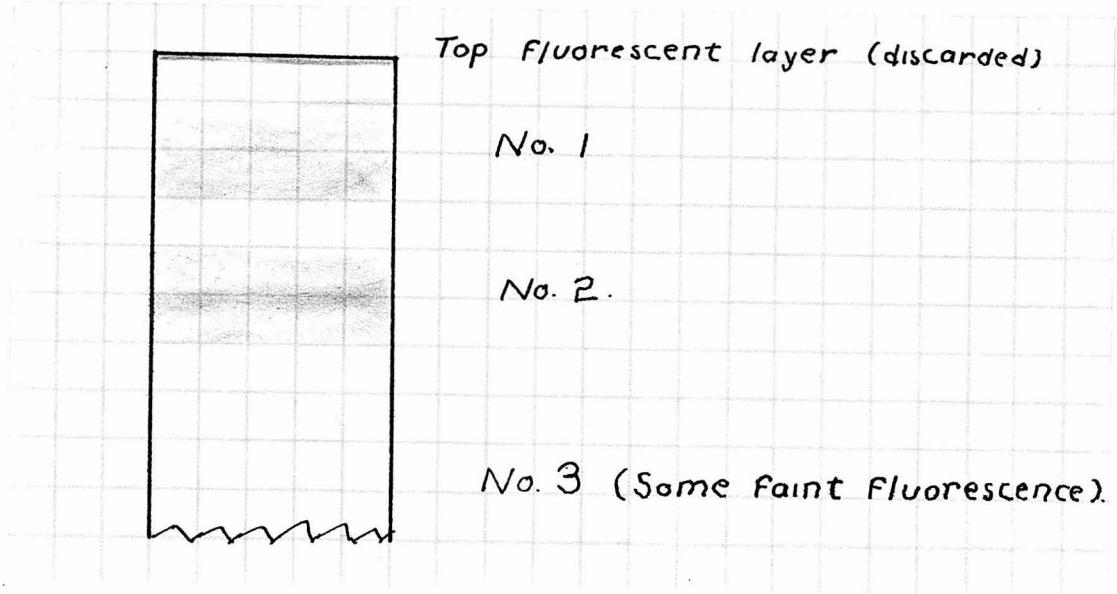
*I should like to thank Professor L. Zechmeister, Dr. A. Polgar and Dr. A. le Rosen for their invaluable suggestions on this part of the problem.

freed from solvent. The residues in each case were taken up in ether, filtered, freed from solvent and dried thoroughly in vacuo over sulfuric acid. In a typical experiment the following results were obtained:

1. Top Zone ..... 0.58 g. (13.6%)
2. Second Zone ..... 1.06 g. (24.8%)
3. Non-fluorescent Zone ..... 0.65 g. (19.9%)
4. Filtrate ..... 1.4 g. (32.8%).

The recovery based on starting material was therefore 92.1%.

Figure I



After several experiments of this type, the collected fractions were separately chromatographed on the same type of column, the size being proportional to the amount of substance to be chromatographed. Each of the main zones mentioned above divided into several zones when developed with 5% ethanol in benzene. Each of these new fractions was eluted as before, collected, and rechromatographed on a new column. Each, as before, separated into several fractions; and at this point, it was decided to collect these substances and attempt to characterize them. In deciding to examine the fractions as they were, without further purification, we were influenced by the fact that the benzene solutions used as stock solutions in the experiment had begun to darken considerably and to smell of ammonia. It may have been this decomposition which prevented us from obtaining a chromatographically pure substance.

The main results of this series of experiments are shown in Table I. From the nature of the experiment, it is quite possible that each zone may contain some of the substance from the zones above and below it, and that many of the adjacent zones may be identical. In Table I, also, is a description of the investigation of each fraction. The results are reported in this manner since the investigation involved literally hundreds of experiments of a similar nature, and since the technique, in each case, was nearly the same.

The investigation was dropped temporarily, since the primary purpose of the problem is an investigation of the structure of sphingosine. However, it has revealed many interesting possibilities

Table I

Main Zone	Column	Speed of Elution	% Recovery	Amt. g.	Appearance	Comments.
		c	50	0.207	light yellow solid	forms ether insoluble dibromide of m.p. 120-121 Found: C, 57.9; H, 10.1; Br. 24.6 (P.L. Nichols Jr.
1		c	60	0.290	light crystalline solid	
		↓	"	0.053	yellow waxy solid	
		↓	"	0.099	light yellow solid	
		↓	"	0.133	yellow oily solid	Gives no acetylation product.
		↓	"	0.143	yellow waxy solid	" " " "
		↓	"	0.104	" " "	" " " "
		↓	90	0.171	light crystalline solid	Acetylation product not investigated.
		↓	"			
2		↓	"	0.497	" " "	Acetylation product of m.p. 106-108°. Found- C, 73.9; H, 11.8; N, 2.3.
		↓	"	0.345	light yellow solid	Acetylation product of m.p. 100-102°. Found: C, 73.0; H, 11.3; N 2.4.
		↓	95	0.287	" " "	Acetylation product of m.p. 100-103°. Found: C, 73.6; H, 11.6; N, 2.2.
		↓	"	1.395	mixed solids.	Acetylation product of m.p. 95-98°. Found: C, 74.1; H, 11.6; N, 2.3.
3		↓	90	0.358	yellow crystal. solid.	Acetylation product not investigated.
		↓	"	0.722	" " "	" " " "
	non Fluorescent			0.820	white crystal. solid	Recrystallized from methanol - m.p. - 145-1 Gives Liebermann reaction and shows no lowering of the m.p. of a known sample of cholesterol.
	Filtrate.				yellow oil	Hydrogenated to give crystalline solid, m.p. 35 Found: C, 76.9; H 12.6. This hydrolyzed to give stearic acid, m.p. 6 Found: C, 75.6; H, 12.9. Neutral Equivalent - 297. M.p. of amide - 90-91°

for future investigations.

The Preparation of Sphingosine from Sphingosine Sulfate.--- Seven grams of sphingosine sulfate was ground in a mortar with 100 ml. of 10% aqueous sodium hydroxide solution. The resulting mixture was extracted in a separatory funnel with ether, and the ether solution was dried over sodium sulfate. Evaporation of the solvent gave 6 g. of a light solid, which was dried *in vacuo* over sulfuric acid.

The Preparation of Triacetyl Sphingosine[14].--- Four grams of sphingosine, prepared as above, was treated in the usual manner with 12 ml. of pyridine and 12 ml. of acetic anhydride. The resulting solution was left at room temperature for twenty-four hours and was then evaporated under reduced pressure to about half its volume. On standing at 5° for twelve hours it deposited crystals. These were filtered and recrystallized from acetone to give 2 g. of the triacetyl compound of m.p. 99-100°. Some samples, after repeated recrystallizations from acetone, had a m.p. of 96-97°.

Anal. Calcd. for $C_{24}H_{43}NO_5$ (425.6): C, 67.7; H, 10.2; N, 3.3. Found: C, 67.2; H, 11.1; N, 3.3.

The Reaction of Triacetyl Sphingosine with Perbenzoic Acid.--- To a solution of 0.6 g. of triacetyl sphingosine in 10 ml. of chloroform was added a chloroform solution containing 0.22 g. of perbenzoic acid (found by titration of the solution with thiosulfate). The solution was allowed to stand at 5° for twenty-four hours and was then

left at room temperature for forty-eight hours. At the end of this period, a thiosulfate titration revealed that the active oxygen in the solution had decreased no more than that in a similar solution containing no triacetyl sphingosine. The solution was refluxed until the perbenzoic acid had decomposed (about one hour) and was then freed from solvent. The residue was taken up in ether, washed with sodium bicarbonate solution and water, and dried over sodium sulfate. On evaporation of the solvent there was obtained an oil, which crystallized on standing. After two recrystallizations from acetone, it melted at 100-101°. The mixed melting point with the starting material was 100-101°.

Bromination of Triacetyl Sphingosine.--- To 0.5 g. of triacetyl sphingosine in 5 ml. of chloroform was added, at 0°, 0.21 g. (a 10% excess over theoretical) of bromine in 5 ml. of chloroform. The bromine was taken up very slowly till about one-third of the solution had been added. At this point the decolorization ceased, and the remainder of the bromine solution was added. After standing overnight at room temperature, the solution was still colored. It was washed with aqueous sodium bisulfite and water and was then dried over sodium sulfate. Evaporation of the solvent left an oil, which, after two recrystallizations from acetone, melted at 100-101°.

Anal. Calcd. for $C_{24}H_{43}NO_5Br_2$ (565.4): C, 51.0; H, 7.7; Found: C, 67.2; H, 10.8.

The Reaction of Triacetyl Sphingosine with Silver Iodobenzoate.---
To a suspension of silver benzoate in 15 ml. of dry benzene was added, in portions with shaking, 0.65 g. of iodine in 10 ml. of dry benzene. To this pink suspension was added, slowly with shaking, a solution of 1 g. of triacetyl sphingosine in 9 ml. of dry benzene. The red color disappeared, and the thick pasty mixture became more fluid. The suspension was refluxed for one hour and was then treated with a pinch of sodium bisulfite, filtered, and freed from solvent. After two crystallizations of the resulting residue with acetone, a crystalline compound of m.p. 100-102° was obtained. The mixed melting point with the starting material was 100-102°.

Anal. Calcd. for $C_{38}H_{52}O_9N$ (666.8): C, 68.4; H, 7.9.
Found: C, 68.2; H, 10.5.

The Reaction of Triacetyl Sphingosine with Potassium Permanganate.---
A solution of 0.2 g. of triacetyl sphingosine and 0.2 g. of potassium permanganate in 25 ml. of acetone containing a few drops of water was refluxed for three hours in an atmosphere of carbon dioxide. At the end of this period the solution was still somewhat colored, but considerable manganese dioxide had precipitated. The color was discharged with a few drops of formalin, and the manganese dioxide was removed by filtration. On partial evaporation of the solution, a crystalline precipitate was obtained, which melted at 100-102°. A mixed melting point with the starting material was 100-102°.

The Reaction of Triacetyl Sphingosine with Hydrogen Peroxide[15].--- A solution of 0.2 g. of 30% hydrogen peroxide in 1 ml. of acetic acid was heated at 80-85° for one hour and was then allowed to cool to room temperature. To this solution was added 0.2 g. of triacetyl sphingosine, and the mixture was warmed on the steam-bath until a clear solution was obtained. On cooling, the solution deposited crystals, which were filtered, washed with water and recrystallized from dilute acetic acid to give needles of m.p. 96-97°.

Anal. Found: C, 67.2; H, 11.1; N, 3.3.

A mixed melting point of this compound with a sample of triacetyl sphingosine of m.p. 95-97° produced no lowering.

Hydrolysis of the Low-Melting Triacetyl Sphingosine.--- The triacetyl sphingosine obtained in the above experiment (0.1 g.) was refluxed in 5 ml. of methanolic hydrogen chloride for twenty-four hours. To the resulting alcoholysate was added water and dilute sodium hydroxide solution until the solution was alkaline to litmus. The resulting precipitate was extracted from the alkaline solution with ether, and the ether solution was washed with water and dried over sodium sulfate. The solvent was evaporated, and the crystalline residue was dissolved in methanol. It showed no optical activity.

At the time of writing, research is being carried out along these and other lines; but because of the necessarily incomplete nature of the results obtained, they are not included in this report.

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15. Scanlan and Swern, J. A. C. S., 62, 2305 (1940).

Part VI

The Longitudinal Muscles of the Holothurians (a Summary)

In connection with the research program in my minor department, animal physiology, I investigated the length muscles of the holothurians. The following is a summary of the main points of the research.

The tonic properties of the muscle were first investigated. It was found that the muscle possessed considerable tone, and that it exhibited a type of reflex contraction when suddenly stretched as by a weight. The fact that it did not exhibit any "snowplow" effect is, I think, unique in the field of the physiology of the musculature of this and related classes of animals.

The muscle was also found to contract quite quickly under electrical stimulation and to relax quickly and completely after the contraction. This, too, is not usual for animals of this class.

In conclusion, it might be said that these muscles behave as if they performed many of the functions of both smooth and striated muscles of vertebrates.

Summary

Part I

A new method for the determination of the structure of polysaccharides has been proposed. A method for the benzylation of sugars has been found, since the benzylation of the polysaccharides is necessary as the first step in the proposed method.

Part II

In connection with the physiological action of organic fluorine compounds, a number of fluorinated amino acids have been prepared. The synthesis of 3-fluoro-dl-tyrosine, 3-fluoro-5-iodo-dl-tyrosine, 3-fluoro-dl-phenylalanine, 3'-fluoro-dl-thyronine, 3'-fluoro-3,5-diiodo-dl-thyronine and 3'-fluoro-5'-iodo-3,5-diiodo-dl-thyronine has been described.

In connection with the synthesis of 3,5-difluoro-dl-thyronine, the preparation of the necessary starting materials has been described.

Part III

The synthesis of dl-3,5-diiodo-4-(3',5'-diiodo-2'-hydroxyphenoxy)-phenylalanine, an isomer of thyroxine, is described. This compound is physiologically active, the activity being approximately one twenty-fifth of that of dl-thyroxine. This finding is in accordance with a prediction relating thyroxine-like activity to chemical structure.

The attempted synthesis of dl-diiodo-4-(3',5'-diiodo-4'-aminophenoxy)-phenylalanine is described.

Part IV

The separation of the carotenoid, β-carotene, from bovine spinal cord is described.

Part V

The preparation of large amounts of cerebroside-sphingomylin fraction from bovine spinal cord is described. The preparation of sphingosine and sphingosine derivatives, and reactions proposed to lead to a determination of the structure of sphingosine are given.

Propositions

Propositions for the Ph. D. Examination

I

The strong para-directing effect of the halogens in the benzene ring can be explained qualitatively on the basis of the inductive and resonance effects.

Within the group, it can be determined quantitatively by a simple empirical relation.

II

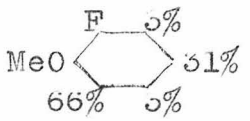
In 1926 Ingold gave values for the relative directive powers of fluorine and methoxyl derived from nitration of fluoroanisole.

I propose that these values are not reliable, since it can be shown that his compounds contained no fluorine.

E.L. Holmes and C.K. Ingold, J.C.S. 129, 1328 (1926)

III

In the article mentioned in proposition II, Ingold stated that the relative amounts of the various isomers formed in the nitration of fluoroanisole are as follows:

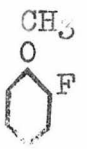


I propose that the amount of 4-nitrofluoroanisole formed in this reaction is in reality 51% and that the method used

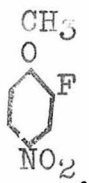
namely, chromatography of the reaction mixture on a suitable adsorbent, with isolation of the products, is an exact quantitative method for such a determination. It could be applied in many cases for recalculation of exact values of the directive influences of various groups in the benzene ring.

IV

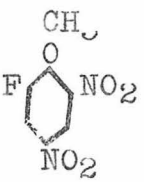
The melting-points of aromatic nitro-compounds show many apparent anomalies, such as the series:



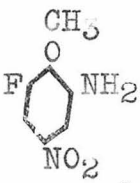
b.p. 154°



m.p. 105°



m.p. 9°



m.p. 109°

Many of these can be explained, and many predictions can be made by a consideration of the effect of the various substituents on certain properties of the nitro group. These properties are those which lead to hydrogen bond formation between molecules or to an electrostatic attraction.

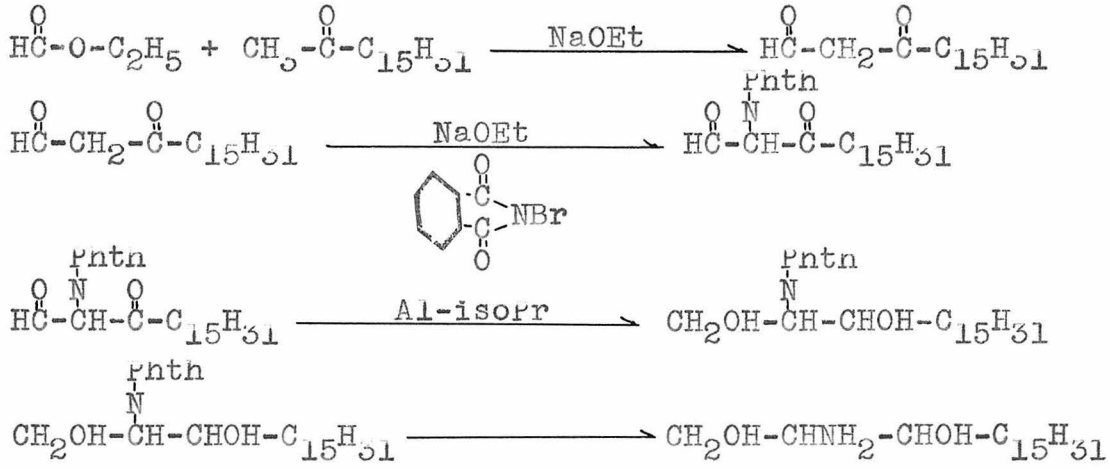
V

For chlorination of the olefinic double bond in easily oxidizable compounds, as the sterols, or for preferential chlorination of one double bond in the presence of others, the usual reagents, as gaseous chlorine or sulfuryl chloride are not practical. I propose the use of phenyliododichloride for such chlorinations.

Willgerodt, Ber. 25, 3499 (1892)

VI

Since sphingosine may be a 1,3-dihydroxy-2-amino-octadecene, I propose the following series of reactions as a synthesis of inactive 1,3-dihydroxy-2-amino-octadecane, which may be inactive dihydrosphingosine:



VII

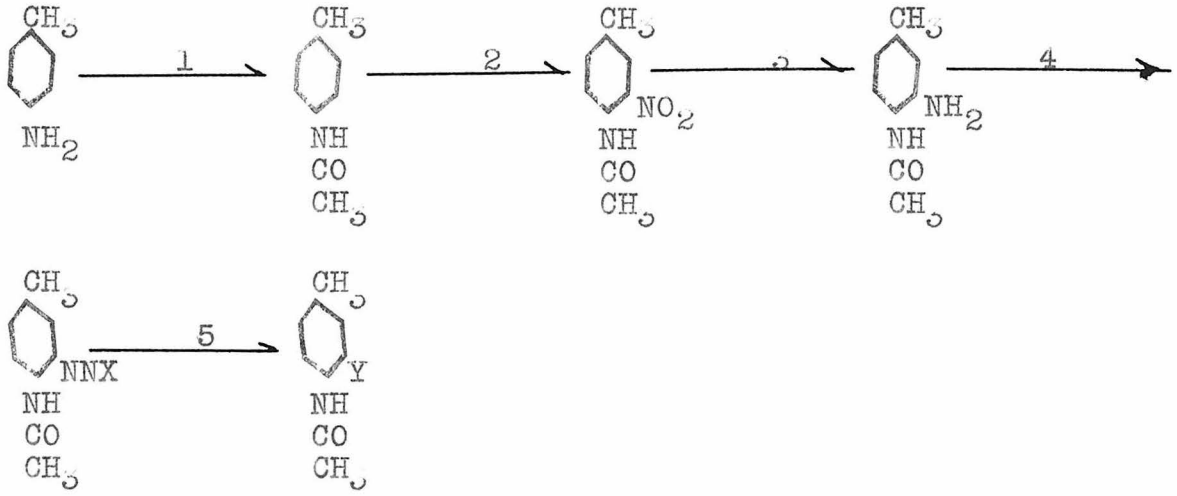
In the foregoing thesis (p. 67) I reported the isolation from the hydrolysis products of spinal cord of compounds which gave acetyl derivatives of the general formula $C_{38}H_{71}O_5N$. These compounds may be amides formed by a reaction between sphingosine and the fatty acid esters known to be present in the solution.

VIII

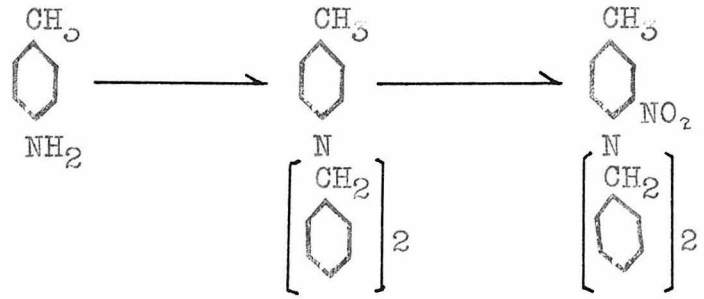
The low yields often obtained in the preparation of amino acids by the Erlenmeyer synthesis can be explained by phosphorylation of the amino nitrogen or of the hydroxyl oxygen, if present.

IX

Reactions involving the following type of blocking group, though apparently possible and often attempted, are not practical.



In step 3, the product of a catalytic reduction is a methyl benzimidazol, while in step 4, the product is an acetyl benztriazol*. As a means of avoiding these difficulties, I propose the following alternative series of reactions:



etc.

* See thesis, p. 17.

X

H.J. Jordan says that the length muscles of the holothurians are similar to the skeletal muscles of the vertebrates,

and that it is not known whether many of these properties are present in the normal animal or whether they originate from injury incurred during their preparation.

The truth may be that some of these properties are present in the intact animal while some are due to injury.

XI

The longitudinal muscles of the holothurians show a type of stretch reflex. This reflex is similar in appearance to the stretch reflex found in mammals, but differs in most other respects.

XII

A course which would present a survey of the main types of practical organic reactions with specific examples of the types of compounds formed by their use would be of great benefit to organic chemists engaged in research.