SOME ORGANIC FLUORINE COMPOUNDS OF THERAPEUTIC INTEREST

By

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fulfillment of the requirements for
the degree of Doctor of Philosophy

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INTRODUCTION

The lack of studies on the physiological properties of organic fluorine compounds coupled with the interesting findings of Midgely and Henne (1) and of Henne (2, 3) with regard to the low toxicity of fluoroform and dichloro-difluoromethane (Freon 12) stimulated an interest in the therapeutic possibilities of other fluorine derivatives. The purpose of the present investigation was to study the effect of the introduction of the fluorine atom into the nucleus on the antiseptic properties of a series of mercurials. Suter (4) has prepared a series of p-fluoro-o-alkylphenols and has studied their antiseptic properties. However, no work has been published on the preparation and bactericidal effects of fluorinated mercurials. In fact, with the exception of 2-fluoro-3-acetoxymercuri-4-amino-5-chlorotoluene and 2-fluoro-3-iodomercuri-4-amino-5-chlorotoluene which were prepared as intermediates in a reaction series (5) and the mercury derivatives of benzotrifluoride which also contain a nitro, hydroxy or carboxy group on the ring (6), no other fluorinated aromatic mercurials could be found in the literature.

In the course of the work, meta and para-fluoroacetanilids were prepared for identification purposes. Since they are derivatives of acetanilid, it seemed desirable to study them pharmacologically. This work has also been included. Some preliminary bacteriological studies on the fluorinated aromatic mercurials has been carried out and is reported.

CHEMISTRY

Literature Survey

The preparation of all types of organic fluorine compounds is comprehensively reviewed by Bockemüller (7). Among the early methods for the preparation of aromatic fluorine compounds is the heating of the diazopiperide with concentrated hydrofluoric acid (8). The method is both cumbersome and dangerous due to the instability of the diazopiperide. Valentiner and Schwarz (9) described the preparation of aromatic fluorine compounds by heating the diazonium chloride solution with 70% hydrofluoric acid. Swarts (10) modified the method slightly in that he diazotized the proper amine in 70% hydrofluoric acid and then heated the solution of diazonium fluoride. In 1927, Balz and Schiemann (11) reported the preparation of fluorobenzene and several other aromatic fluorine compounds by the thermal decomposition of the stable dry borofluoride. In a series of papers appearing since 1927 (12 to 32), Schiemann and coworkers have extended the reaction to the preparation of a wide variety of aromatic fluorine compounds.

The direct fluorination of aromatic compounds with fluorine gas is unsatisfactory (7). Bigelow and coworkers (33, 34) have described the fluorination of aromatic compounds with fluorine gas but did not isolate definite compounds. When hexachlorobenzene was used, partially or completely saturated chloro-fluorocyclohexanes were obtained, a total of twelve compounds being separated (35). Whearty (36) and Bancroft and Whearty (37) have isolated various chloro-fluorobenzenes from the reaction of hexachloro- and sym-trichlorobenzenes with fluorine gas. The

action of fluorine on benzoic acid leads to mixtures (38). When acetanilid was treated with lead tetrafluoride, some p-fluoroacetanilid was obtained (39). Gottlieb (40) prepared 2,4-dinitro-fluorobenzene from 2,4-dinitro-chlorobenzene and potassium fluoride. However, this type of preparation is quite limited. For our purposes, the method of Schiemann was considered most satisfactory.

A large number of aromatic compounds containing mercury in the nucleus has been reported in the literature. The customary method of preparing these mercurials consists in heating the aromatic compound with a solution or suspension of mercuric chloride, mercuric acetate or mercuric oxide in water, glacial acetic acid or some other medium. The mercury is introduced directly into the ring. In most cases therefore, the mercurial is contaminated with one or more possible isomers or disubstitution products (41-43). Some mercurials can not be prepared by this method. Less common methods of preparation are: the reaction of sodium amalgam on aryl halides (44), the reaction of mercury halides with Grignard solutions (45), and the reaction of mercuric chloride and aryl sulfinic acids (46). McClure and Lowy (47) succeeded in preparing mercury derivatives of aromatic compounds by treating the corresponding diazonium chloride solution in the cold with finely divided mercury produced by very rapid stirring with a specially constructed stirrer.

Nesmejanow (48) developed a method of preparing aromatic mercury derivatives of those nuclei containing no substituents or negative groups such as $-CH_3$, $-OCH_3$, $-OC_2H_5$, etc., from the diazonium chloride-mercuric chloride double salts. He diazotized the amines in concentrated hydrochloric acid and treated the solution with a solution of mercuric chlo-

ride in concentrated hydrochloric acid to obtain the double salt. In those cases where the diazonium chloride was insoluble and relatively stable when dry, he mixed the dry diazonium compound with an equivalent amount of mercuric chloride. The replacement of the diazonium group by the -HgCl group was effected by shaking the double salt with finely divided copper in some non-aqueous liquid at room temperature until the evolution of nitrogen ceased. A few years later, he succeeded in modifying his method (49) so as to allow the preparation of mercury derivatives of nuclei containing substituents such as -COOH, $-NO_2$, halogens, etc. The double salt or the mixture of the diazonium salt and mercuric chloride was stirred with catalytic copper in a variety of media at temperatures ranging from 0° to -70° C. The low temperature was necessary to retard undesirable side reactions due to the instability of the diazonium chlorides at room temperatures.

Dunker, Starkey and Jenkins (50) have described the preparation of some mercurials from the diazonium borofluoride. The latter, after being purified, was mixed with one equivalent of mercuric chloride and then slowly added to a suspension of stannous chloride and freshly reduced mercury in acetone. The reactions were carried out at room temperature; and, in those cases where comparison was possible, the yield and quality of the products compared favorably with those obtained by Nesmejanow.

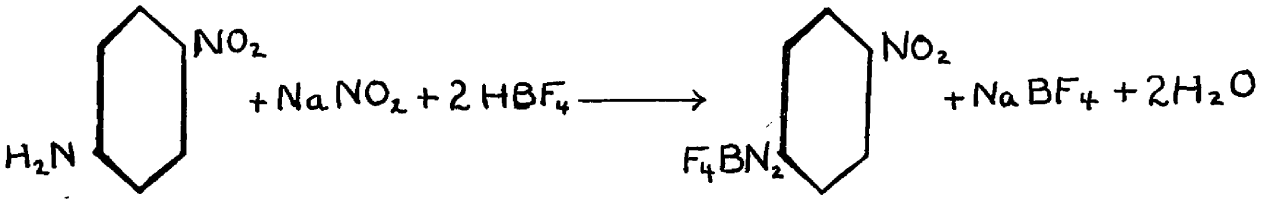
All of the compounds described in this paper were prepared by existing methods adapted to the case in question. The diazonium borofluorides were prepared as described by Dunker, Starkey and Jenkins (50) and the fluoro-substituted compounds prepared from the borofluorides as described by Schiemann and Pillarsky (15). The mercurials were prepared

as described by Dunker et al (50) or by direct mercuration. References to the preparation of individual compounds will be mentioned specifically later.

Experimental

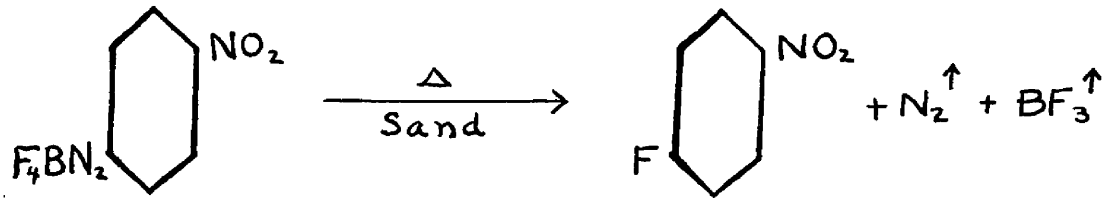
p-Fluorophenylmercuric chloride was prepared by the following series of reactions. Since many of these reactions were used in preparing related compounds, they will be described in detail here and later referred to by number.

I Preparation of p-Nitrophenyldiazonium Borofluoride



The method is that given by Balz and Schiemann (11) as modified by Dunker, Starkey and Jenkins (50). To 420 cc. (2.5 mols) of 50% borofluoric acid in a 1 l. beaker was added 138 g. (1 mol) p-nitraniline while stirring. Most of the amine dissolved and recrystallized on cooling the solution in an ice bath. While stirring vigorously, a solution of 69 g. (1 mol) sodium nitrite in 150 cc. water was added slowly from a separatory funnel, keeping the temperature of the mixture about 5°. When the addition was complete, the solid was filtered on a fritted glass funnel, washed once with alcohol and then three times with ether and dried partially by drawing air through it. The resulting light yellow solid was dried over night in a desiccator. The yield was 237 g. or the theoretical quantity. This preparation was repeated several times, the yield always being practically quantitative.

II Preparation of p-Nitrofluorobenzene



The method is that given by Schiemann and Pillarsky (15). About 40 g. of p-nitrophenyldiazonium borofluoride was mixed with approximately three times its bulk of sand and the mixture divided in two. Each half was placed in a 500 cc. pyrex distilling flask having the delivery tube bent so that the flask could be clamped on its side. The flask was then connected to the condenser and the compound received in a cooled 500 cc. filtering flask to which suction was applied by means of a water pump, a trap containing a solution of sodium hydroxide being inserted between the pump and the receiver. The flask was heated slowly, beginning near the surface of the solid and working down as the compound was decomposed. When the decomposition was complete, the flask was heated strongly to drive off the last of the nitrofluorobenzene. After cooling, the sand was shaken out and used to dilute the succeeding run of diazonium compound. The distillate was shaken with several portions of a saturated aqueous solution of sodium carbonate to remove any dissolved boron trifluoride and the separated oily layer steam distilled. There was obtained 79 g. of pale yellow oily liquid and an additional 3 g. on steam distilling the sodium carbonate washings, giving a total of 82 g. or 58% of the theoretical.

For preparing large quantities of p-fluoronitrobenzene, this method is somewhat tedious and efforts were made to modify it with the hope of producing satisfactory yields with less inconvenience. Several attempts

to prepare fluoronitrobenzene by decomposition of the diazonium compound in inert liquids were made.

A 5 g. portion of the diazonium compound was refluxed in 100 cc. xylene. The compound was insoluble. It slowly darkened as boron trifluoride escaped from the condenser. When the decomposition was complete, the flask was connected to a fractionating column and most of the xylene removed. The tarry residue was then steam distilled and a small amount of pale yellow liquid separated. The last traces of xylene were removed from it by careful evaporation leaving a few drops of pale yellow oil which was not identified because of the small amount available. A similar trial in kerosene gave a like result.

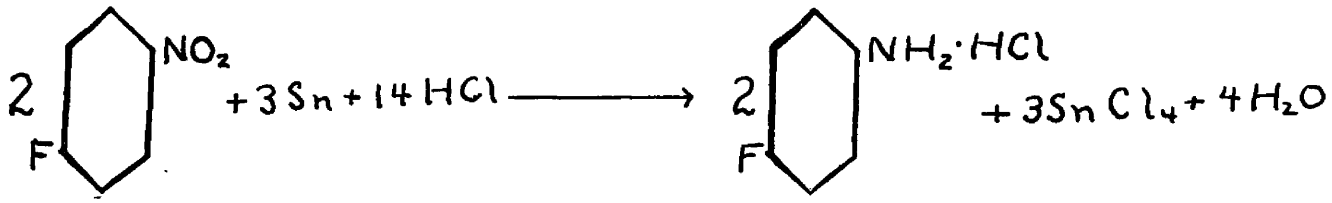
It was thought that if the diazonium compound were introduced in small amounts into a liquid kept at a temperature just above the decomposition point of the diazonium compound (150-160° for p-nitrophenyldiazonium borofluoride), the incidence of side reactions might be reduced. The dry diazonium compound was slowly added to melted acetamide kept at 160° in a flask equipped with a stirrer. When all had been added, the mixture was steam distilled and the oily layer separated. In order to facilitate the identification of the nitro compound obtained, it was reduced to the amine with tin and hydrochloric acid and the amine acetylated. The acetyl compound was crystallized once from dilute alcohol and melted 112° (m.p. acetanilid 114°; p-fluoroacetanilid 152°). Only a small amount of nitro benzene was obtained in the reaction.

In another trial, 100 g. (0.42 mol) of the diazonium compound was mixed with an equal bulk of 200 mesh silica gel and then with twice its bulk of sand and slowly heated in a 2 l. flask immersed in an oil bath

and connected to a 1 l. round-bottomed flask to act as a trap. The heating was continued until no more boron trifluoride was evolved. The residue in the flask was extracted with two portions of warm ether, the ether removed by distillation and the residue steam distilled. A small yield of p-fluoronitrobenzene (identified as p-fluoroacetanilid melting 152°) was obtained.

None of the modifications was deemed satisfactory, and all of the necessary p-nitrofluorobenzene was prepared as first described.

III Preparation of p-Fluoroaniline



The reduction of the nitro compound was carried out by means of the customary tin and hydrochloric acid method. To 150 g. (excess) tin and 100 g. (0.7 mol) p-fluoronitrobenzene in a 2 l. round-bottomed flask was added with agitation and cooling when necessary, 300 cc. (excess) hydrochloric acid. When the addition was complete, the mixture was refluxed for 30 minutes and then set aside to cool. The contents of the flask were diluted with 300 cc. of water and a solution of 270 g. sodium hydroxide in 450 cc. water added. The mixture was steam distilled and the amine separated. The aqueous distillate was saturated with sodium chloride and extracted with ether, the ether layer separated, dried, and the ether distilled off on a water bath. The combined p-fluoroaniline weighed 58 g. representing 75% of the theoretical. The yields were uniformly 75 to 85%. The amine oxidized rather rapidly, turning red, then

brown. A sample which was pale yellow when sealed in an ampul also darkened in a very short time.

p-Fluoroaniline was also prepared by the catalytic reduction of p-nitrofluorobenzene using palladium catalyst and hydrogen at room temperature and a pressure slightly above atmospheric. The palladium catalyst was prepared by mixing 3 g. charcoal with 50 cc. water in the hydrogenation flask and adding 3 cc. of a solution containing 0.1 g. palladium chloride per cc. The flask was connected to the hydrogenating apparatus and shaken for one-half hour at which time no more hydrogen was being taken up. The contents of the flask were filtered through a Buchner funnel and drained dry. The catalyst was returned to the flask and 100 cc. 95% alcohol and 7 g. (0.05 mol) p-fluoronitrobenzene added. The flask was connected to the apparatus and shaken for 2 hours until no more hydrogen was taken up. Three and seventy-eight hundredths l. hydrogen were used (theoretical 3.56 l.). The mixture was filtered through a Buchner funnel and the alcohol distilled off. The yield was practically quantitative. The acetyl derivative was prepared using a 1.0 g. sample and, after one crystallization from dilute alcohol, melted at 153°. A mixed melting point with a sample of p-fluoracetanilid prepared by the tin and hydrochloric acid method gave no depression of the melting point.

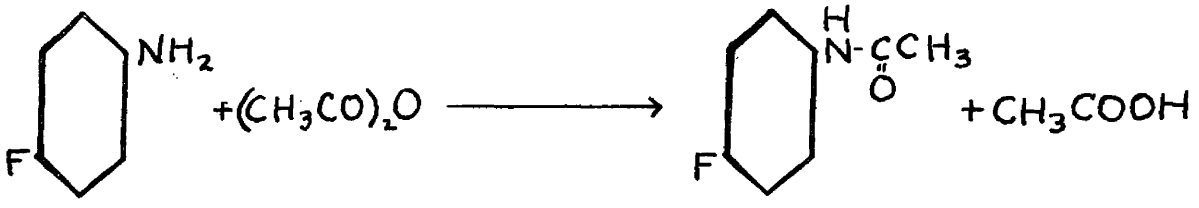
The above catalytic reduction is of interest because of the variance in behavior of organic fluorine compounds on reduction as reported in the literature. The following statements were made by Swarts (51):

> All of the aliphatic compounds of fluorine which I have studied are refractory to hydrogenation in the presence of platinum black at

> ordinary temperatures while the corresponding chlorine derivatives are easily reduced. On the other hand the aromatic fluorine compounds lose hydrofluoric acid and one obtains derivatives of cyclohexane. Thus fluorobenzene gives cyclohexane, fluorotoluene gives methylcyclohexane, fluorobenzoic acid gives hexahydrobenzoic acid and fluoroaniline gives cyclohexylamine.

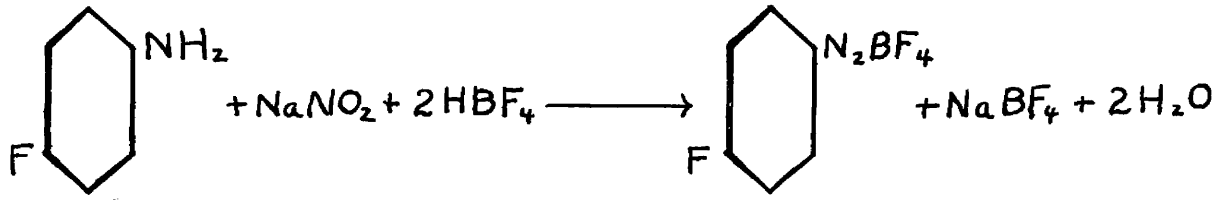
Swarts later reported (52) that in the reduction of fluorobenzene in the presence of platinum black, fluorine is first removed as HF and the ring is then reduced to cyclohexane. H. L. Hanson (53) reported that attempts to reduce several halogenated (chlorine and fluorine) amino ketones to the secondary alcohols using hydrogen and platinum catalyst either failed to give the desired products or produced small amounts of compounds which it was not possible to identify. He suggested that the halogen was removed and the C to N bond split. Suter, Lawson and Smith (4) on the other hand, found the C to F bond stable to hydrogenation in the presence of platinum black.

IV Preparation of p-Fluoroacetanilid



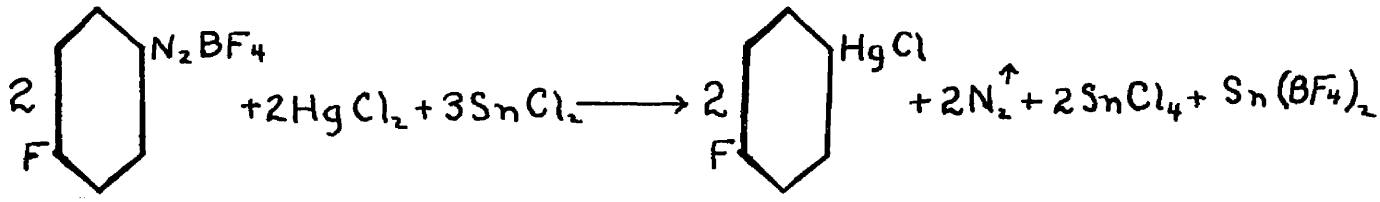
The acetyl derivative was prepared by treating the amine with acetyl chloride or acetic anhydride in excess. The excess acetylating agent was decomposed with water and the solid product crystallized from dilute alcohol. p-Fluoroacetanilid formed long, slender, white needles melting at 153°. The following melting points are reported in the literature: 150-151°, (54); 150°, (55); 150-151°, (15).

V Preparation of p-Fluorophenyldiazonium Borofluoride



The diazotization of 22 g. (0.2 mol) of p-fluoroaniline in the manner described in I gave 36 g. of pale salmon colored silky needles representing a yield of 86%. This yield was duplicated in subsequent trials.

VI Preparation of p-Fluorophenylmercuric Chloride



The diazonium group was replaced with mercury according to the procedure described by Dunker, Starkey and Jenkins (50). The method did not work as well as expected, and many trial runs were made. Difficulties in filtration were encountered and it was necessary to separate the solid by centrifugation. In two trial runs, copper was substituted for stannous chloride. One was carried out in acetone and the other in dioxane. In neither case did the reaction proceed at room temperature. On warming the mixtures under a reflux condenser, the diazonium compound decomposed but the yield of mercurial was very poor.

The best method, still leaving much to be desired, gave a 25% yield of the desired mercurial. To a mixture of 6.8 g. (0.025 mol) mercuric chloride and 12.4 g. (0.055 mol) stannous chloride .2 H_2O in 50 cc. acetone and 10 cc. water was added a solution of 5.2 g. (0.025 mol)

p-fluorophenyldiazonium borofluoride in 100 cc. acetone slowly while stirring. The reaction occurred immediately. When all of the diazonium compound was decomposed, the mixture was warmed and centrifuged. The supernatant liquid was decanted and the solid washed twice by centrifuging with small portions of acetone. The extractives were combined and concentrated. A total of 2 g. of shiny plates were obtained, representing a 24% yield. The compound was soluble in acetone, alcohol, glacial acetic acid, slightly soluble in benzene and insoluble in water. After recrystallization from acetone, the material softened at 278° and melted at 284°.

The p-fluorophenylmercuric chloride accumulated from several trials was recrystallized from hot glacial acetic acid. The compound was obtained as small glistening white plates. Some difficulty was experienced in obtaining a sharp melting point[1], the final temperature depending on the rate of heating. On rapid heating a sample melted 293-294°. Apparently the compound decomposed near its melting point since a melted sample on solidifying remelted at 289°. A sample of the mercurial was dried in an oven at 105° and analyzed for mercury by the method of Tabern and Shelberg (57) which was used throughout this paper.

Samples: 0.1778, 0.1598 g.; Weight of HgS 0.1253, 0.1144 g.

Mercury found: 60.71, 60.86%

Calculated for C_6H_4ClFHg 60.59% Hg

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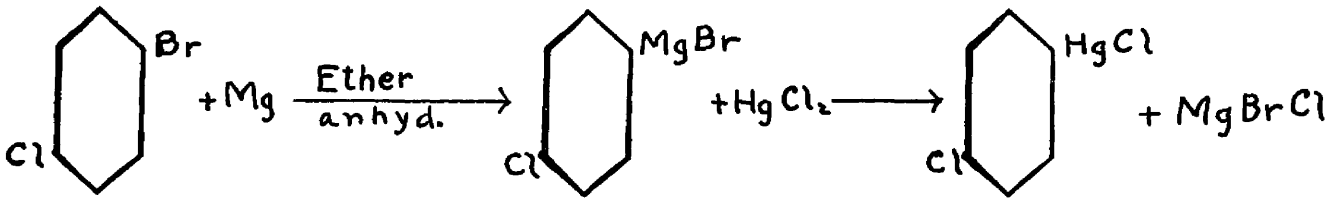
[1]The melting point for p-fluorophenylmercuric chloride given by E. B. Starkey (unpublished) is 285°. Kharasch, Pines and Levine (56) reported 291° for the mercurial prepared by the Grignard method.

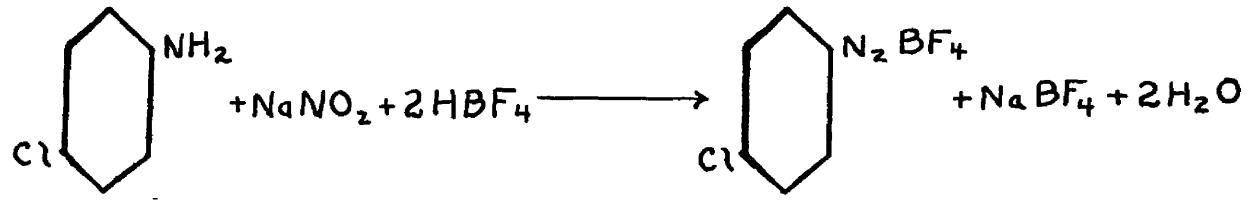
VII Preparation of p-Chlorophenylmercuric Chloride by the Grignard Method



In a 2 l. round-bottomed flask fitted with a stopper carrying a reflux condenser and a separatory funnel was placed 6.0 g. (0.25 mol) magnesium turnings and about 50 cc. anhydrous ether. The condenser was protected from atmospheric moisture and carbon dioxide by a soda lime tube. A solution of 38 g. (0.2 mol) p-chlorobromobenzene in sufficient anhydrous ether was added slowly to the mixture from a separatory funnel as required to maintain the reaction. It was necessary to prime the reaction with a crystal of iodine. After the reaction was complete, the solution was refluxed for 5 minutes and then quickly filtered through glass wool into a 1 l. flask stoppered with a cork carrying a soda lime tube. The theoretical quantity of mercuric chloride (54.3 g. or 0.2 mol) was then added in small portions with shaking. The mixture was diluted to 500 cc. with ether and refluxed for 2 hours. The solid was filtered off and extracted in a Soxhlet extractor with acetone. The warm acetone was then poured into about 800 cc. acidulated water and the solid filtered on a Buchner funnel and partially dried over night in a desiccator. The product was refluxed 1 hour with freshly precipitated silver chloride in a mixture of equal parts of alcohol and acetone. The solution was filtered hot through a steam funnel and allowed to cool. The solid was re-extracted and all filtrates combined and concentrated. A total of 44 g. of mercurial was obtained. The

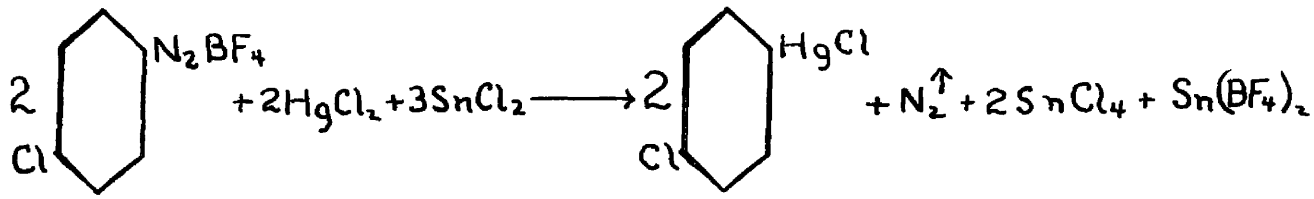
compound melted 231-233°. The following melting points are reported in the literature: 225°, (58); 228°, (59); and 240°, (48). A sample of the material was dissolved in boiling alcohol and refluxed with silver chloride for 2 hours, the liquid filtered hot and allowed to cool. The white plates melted 235.5-237°. The compound was crystallized from boiling alcohol and melted 235-237°. Recrystallization from acetone gave crystals melting at 235-236°. Since further crystalization failed to raise the melting point, this procedure was discarded.

VIII Preparation of p-Chlorophenyldiazonium Borofluoride



To 25.2 g. (0.2 mol) of p-chloroaniline in 70 cc. (0.42 mol) borofluoric acid was added a solution of 14 g. (0.2 mol) sodium nitrite in 28 cc. water slowly in the usual manner. There was obtained 44.5 g. dense white crystals representing a yield of 98% of the theoretical.

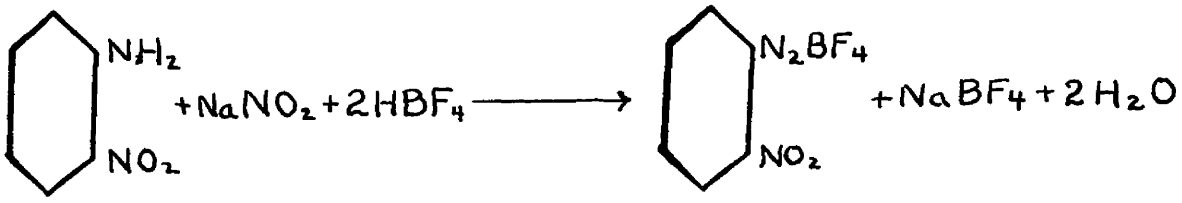
IX Preparation of p-Chlorophenylmercuric Chloride from p-Chlorophenyldiazonium Borofluoride



The replacement of the diazonium group by mercury was carried out in the same manner in which p-fluorophenylmercuric chloride was prepared. A suspension of 33.6 g. stannous chloride 2 H_2O (0.15 mol) and 13.6 g.

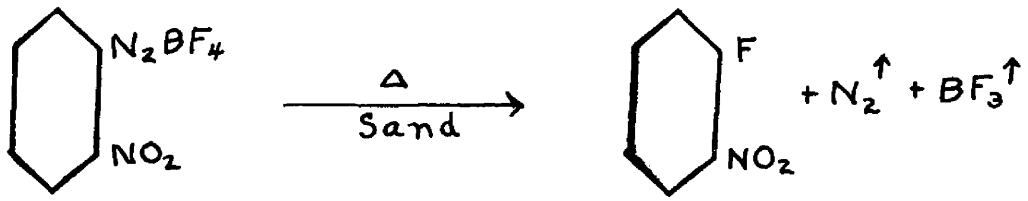
(0.05 mol) mercuric chloride in 100 cc. acetone in a 500 cc. round-bottomed flask was stirred vigorously while slowly adding a solution of 11.3 g. (0.05 mol) p-chlorophenyldiazonium borofluoride and 13.6 g. (0.05 mol) mercuric chloride in 100 cc. acetone. The reaction was instantaneous. At the completion of the reaction, the mixture was refluxed for 10 minutes and poured hot into a bottle and centrifuged. The supernatant liquid was decanted, the solid extracted with two small portions of hot acetone, centrifuged, the extractives combined and the acetone evaporated. The solid was washed free of most of the color with warm ether containing a little alcohol. The residue so obtained melted above 230°. It crystallized from boiling alcohol in small white plates which softened some at 231.5°, clung to the walls of the tube at 234° and melted at 240°. The material was then recrystallized from boiling acetone and came out in small white needles which softened at 238° and melted at 240°. The yield was 4 g. or 24% theoretical.

X Preparation of o-Nitrophenyldiazonium Borofluoride



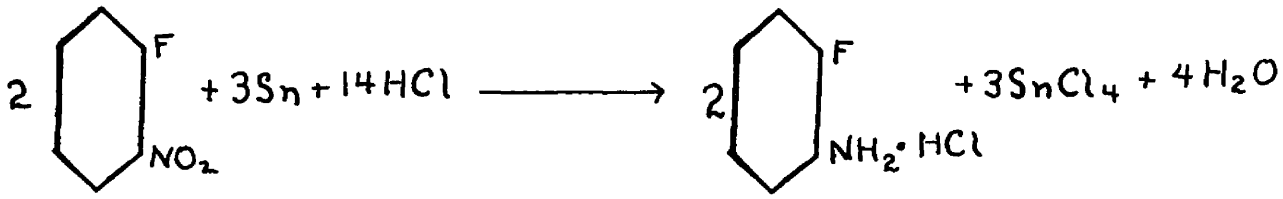
The diazotization of 70 g. (0.5 mol) o-nitraniline (Eastman technical) in the same manner as described in I, gave 109 g. of a dull, yellow-colored powder representing 92.4% of the theoretical.

XI Preparation of o-Nitrofluorobenzene



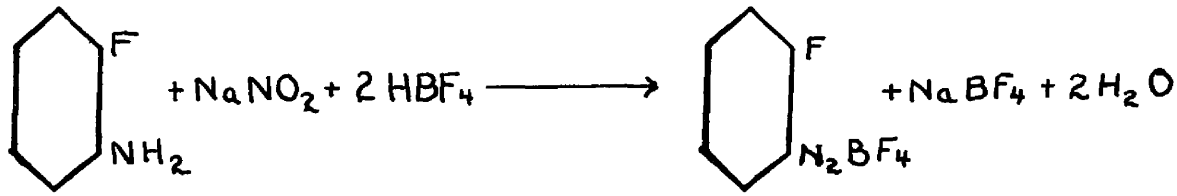
From 109 g. (0.46 mol) o-nitrophenyldiazonium borofluoride using the procedure described in II, there was obtained 8 g. of light orange colored oil representing a yield of 12.5% as compared to 19% reported by Schiemann and Pillarsky (15). This reaction was repeated several times but no increase in yield was obtained.

XII Preparation of o-Fluoroaniline



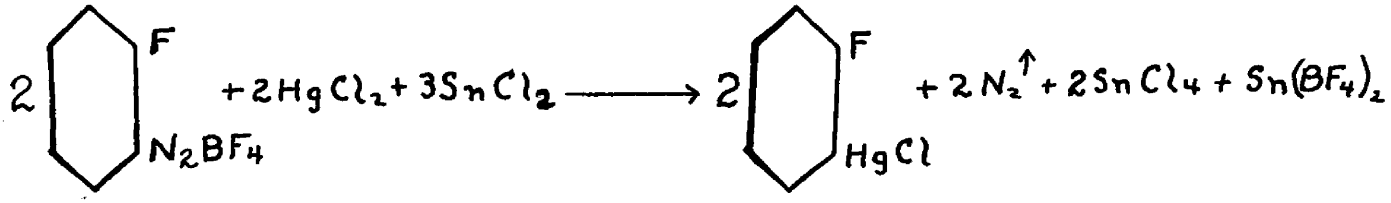
The reduction of 18 g. (0.127 mol) o-fluoronitrobenzene was carried out with tin and hydrochloric acid as given in III and yielded 10 g. of pale yellow o-fluoroaniline or 70% of the theoretical.

XIII Preparation of o-Fluorophenyldiazonium Borofluoride



The diazotization of 10 g. (0.09 mol) o-fluoroaniline as given in I yielded 13 g. of pale yellow, small, soft needles equivalent to 69% of the theoretical.

XIV Preparation of o-Fluorophenylmercuric Chloride



Except for a slight modification, the replacement of the diazonium group was carried out essentially as described in VI. One g. charcoal was mixed with a solution of 10 g. (0.04 mol) mercuric chloride in 50 cc. acetone in a round-bottomed flask and 19 g. (0.084 mol) stannous chloride $\cdot 2H_2O$ added. To this suspension was added with stirring, a solution of 10 g. (0.04 mol) mercuric chloride and 8 g. (0.04 mol) o-fluorophenyldiazoniumborofluoride in 100 cc. acetone. The solid was separated by centrifuging as before, the acetone concentrated, and the crystals obtained washed with ether. About 3 g. of crystals representing 24% of the theoretical was separated and on crystallization from alcohol came out in white needles[2] which softened at 154.5° and melted 156-157°. The crystals gave a positive test for the presence of fluorine (60) a positive test for mercury after decomposing with bromine and no test for mercury without this treatment. Recrystallization of the solid from 50% alcohol did not raise the melting point. When recrystallized from ether, the compound melted 157-160°. The compound was dissolved in ether and precipitated with petroleum ether and now melted 159-160°.

Some of the o-fluorophenylmercuric chloride was dried in a vacuum

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[2]A sample of o-fluorophenylmercuric chloride prepared by the Grignard method was amorphous and melted 90-110° with decomposition (E. B. Starkey, unpublished).

desiccator over P_2O_5 over night and analyzed for mercury.

Samples: 0.1912, 0.3060 g.; Weight of HgS 0.1377, 0.2205

% Mercury found 62.09, 62.12

Calculated for C_6H_4ClFHg 60.59% Hg

Since the analyses were slightly high, the solid was dissolved in 25 cc. hot alcohol and an excess of hot alcoholic solution of calcium chloride added. The fluffy white precipitate was filtered out and the alcoholic filtrate concentrated. The compound was precipitated with water, and washed well with water to remove any calcium chloride. The material was then crystallized from alcohol and melted 157°. It was dried in a desiccator as before and analyzed for mercury.

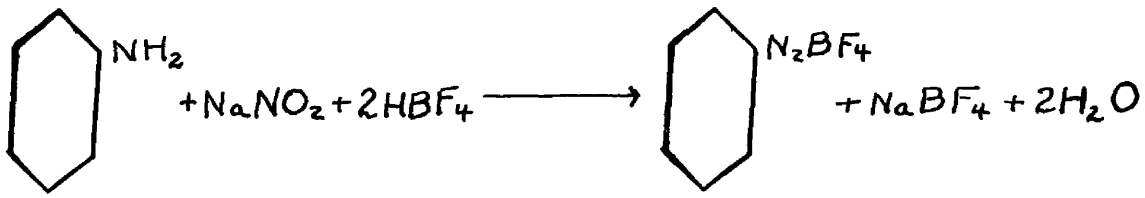
Samples: 0.1577, 0.1945 g.; Weight of HgS 0.1107, 0.1381 g.

Mercury found 60.50, 61.21%

Calculated for C_6H_4ClFHg 60.59% Hg

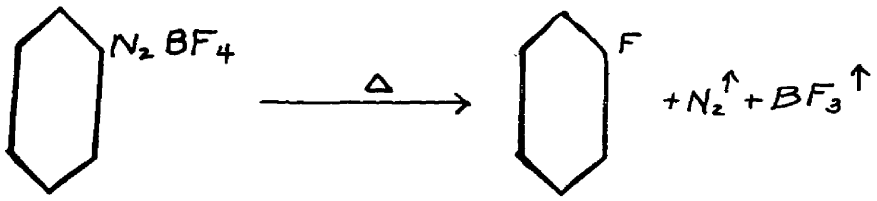
Since only a small amount of o-fluorophenylmercuric chloride was available and since the method was rather tedious, at attempt was made to obtain the compound by the direct mercuration of fluorobenzene.

XV Preparation of Phenyldiazonium Borofluoride



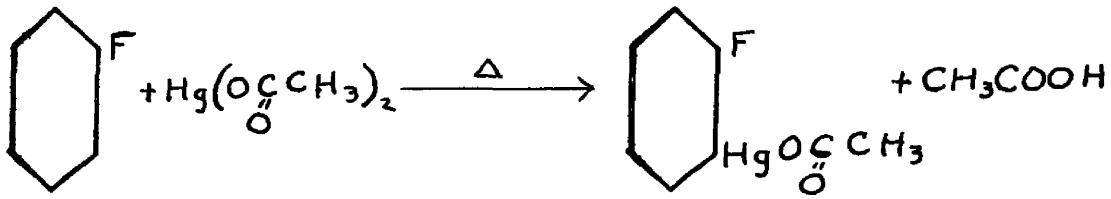
One-quarter mol of aniline (24 g.) was diazotized as given under I yielding 46 g. diazonium compound or 96% of the theoretical.

XVI Preparation of Fluorobenzene



The above diazonium compound was decomposed in 10 g. portions in a 500 cc. distilling flask using a slightly reduced pressure to draw off the gases and cooling the receiver in an ice bath. About 14 g. of somewhat yellow product was obtained, representing a 60% yield of crude product. Balz and Schiemann (11) reported quantitative yields for this decomposition. However, Flood (61) reported a 50 to 56% yield for the preparation of fluorobenzene by this method.

XVII Mercuration of Fluorobenzene

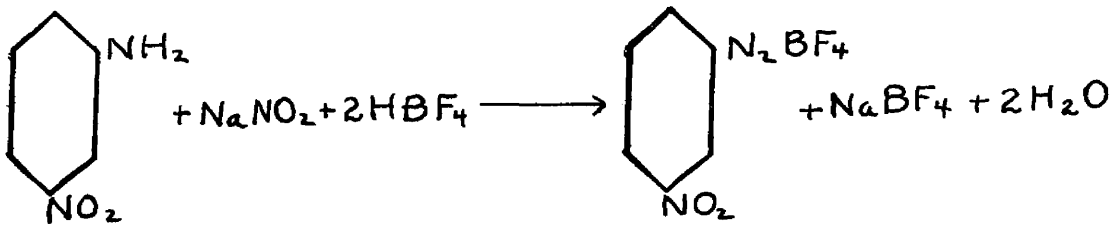


The direct mercuration of fluorobenzene was only partly successful. When 0.094 mol (9 g.) fluorobenzene was refluxed in ethyl alcohol with an equivalent amount of mercuric acetate (30 g.) for 16 hours, no mercurial could be separated.

Another experiment was carried out in glacial acetic acid. A mixture of 20 g. (0.2 mol) fluorobenzene, 65 g. (0.2 mol) mercuric acetate and 100 cc. glacial acetic acid was refluxed for 12 hours. At the end of this time, unused mercuric acetate was still present since a sample when made alkaline with sodium hydroxide gave a yellow precipitate of

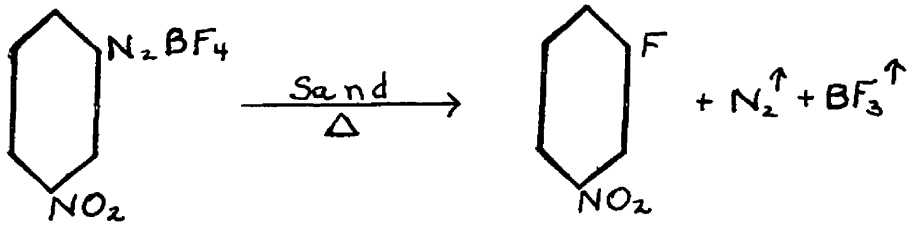
mercuric oxide. The mixture was treated with dilute hydrochloric acid and diluted to about 700 cc. The white amorphous solid was filtered off on a Buchner funnel and partially dried. The mixture was extracted with two portions of hot glacial acetic acid. The acetic acid deposited crystals which melted partially 150-160° and completely by 180°. These crystals were recrystallized several times from ethyl alcohol and a total of 7 g. or about 11% of the theoretical of light needles melting 155-6° and giving no depression of melting point with a sample of pure o-fluorophenylmercuric chloride were separated. The remaining material was amorphous and difficultly soluble in glacial acetic acid, alcohol, and acetone and could not be obtained in crystalline form. It melted over a wide range of temperature and was not identified.

XVIII Preparation of m-Nitrophenyldiazonium Borofluoride



One-half mol or 70 g. m-nitraniline was diazotized as given under I and yielded 110 g. of pale tan crystals or 92.5% of the theoretical.

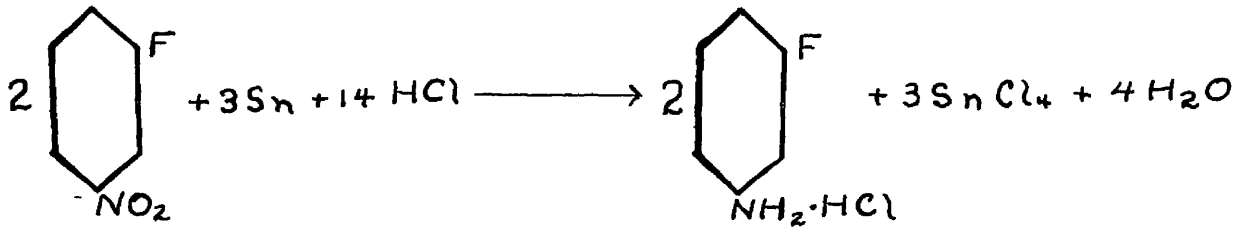
XIX Preparation of m-Nitrofluorobenzene



The above diazonium compound was decomposed as given under II to yield a total of 30 g. m-nitrofluorobenzene or 43% of the theoretical.

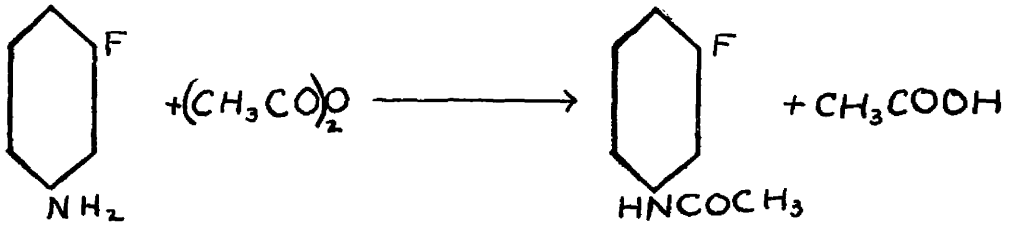
Schiemann and Pillarsky (15) reported 54%.

XX Preparation of m-Fluoroaniline



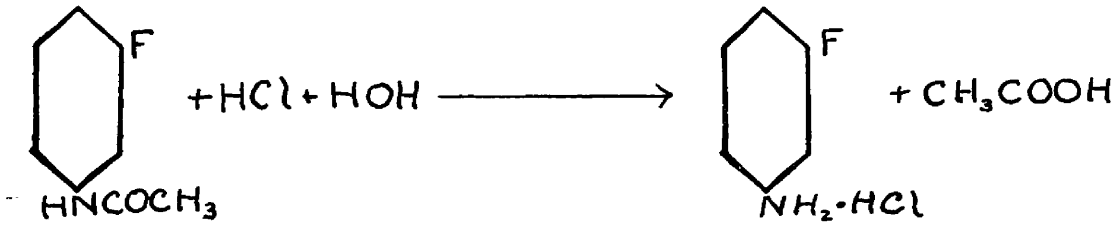
The entire amount of m-nitrofluorobenzene from above was reduced with tin and hydrochloric acid as given under III to give 21 g. m-fluoroaniline or 89% of the theoretical.

XXI Preparation of m-Fluoroacetanilid



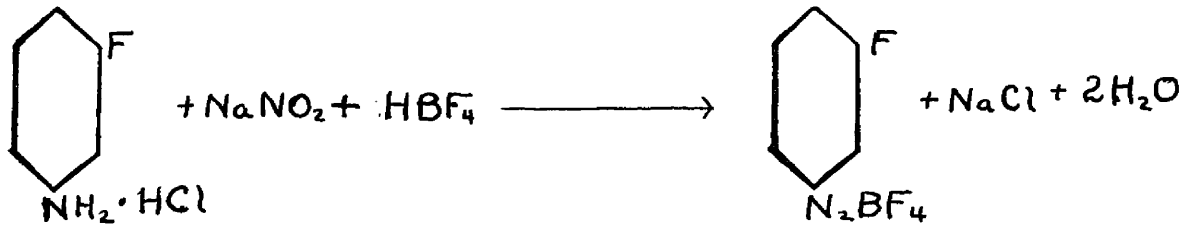
To 20 g. (0.18 mol) m-fluoroaniline was added 20 g. (0.20 mol) acetic anhydride. On cooling, the mixture solidified. The mass was broken up and washed with water. It was dissolved in hot alcohol, water added to the point of cloudiness, and the solution allowed to cool slowly. An oily liquid settled out. It was put in the refrigerator but did not crystallize. When the solution was seeded with a crystal of the acetyl compound, the solution separated a mass of small needles, which, when filtered out and dried, weighed 23 g. or 84% of the theoretical. The compound melted at 86°. Swarts (62) reported 84.6°; Schiemann and Pillarsky (15) reported 84.5°.

XXII Recovery of m-Fluoroaniline



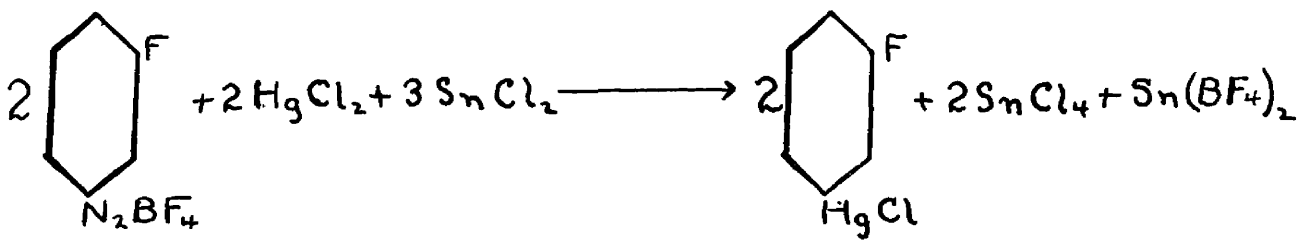
The m-fluoroacetanilid was prepared for use in the experiments of antipyretic activity which are to be described later. When this work was complete, the acetanilid was hydrolyzed to recover the m-fluoroaniline. The remaining 20 g. (0.13 mol) m-fluoroacetanilid in 75 cc. alcohol was refluxed with 50 cc. conc. hydrochloric acid for 3 hrs. On cooling, only a few crystals of the hydrochloride settled out. The solution was concentrated and a crop of crystals removed. On further concentration, a total of 17 g. of hydrochloride was obtained, representing 97% of the theoretical.

XXIII Preparation of m-Fluorophenyldiazonium Borofluoride



The entire amount of amine hydrochloride was diazotized as given in I and yielded 24 g. of pale tan powder which darkened fairly rapidly in the desiccator. The yield was 99% of the theoretical.

XXIV Preparation of m-Fluorophenylmercuric Chloride



The preparation was carried out exactly as described under XIV. From 8 g. (0.04 mol) of diazonium compound there was obtained 3.5 g. of shiny plates equivalent to 28% of the theoretical. On crystallization from boiling alcohol, the crystals melted[3] at 250-251°. The crystals were dried over night in a vacuum desiccator over P_2O_5 and analyzed for mercury.

Samples: 0.2262, 0.2190 g.; Weight of HgS 0.1583, 0.1534 g.

Mercury found 60.91, 60.39%

Calculated for C_6H_4ClFHg 60.59% Hg.

TABLE I

COMPARISON OF THE MELTING POINTS OF HALOGENATED PHENYLMERCURIC CHLORIDES

Halogenphenylmercuric chloride	MELTING POINT ortho	meta	para
fluoro-	159-160	250-251	293-294
chloro-	145[a]	210[a]	225[a], 236[c], 240[b]
bromo-	155[a]	198[a]	249.5[b], 250[a]
iodo-			272.5[b]

[a]M. E. Hanke, J. Am. Chem. Soc., 45, 1326 (1923) (sulfinic acid method)

[b]A. N. Nesmejanow, Ber. 62, 1016 (1929) (diazonium method)

[c]Grignard method, see VII p. 14

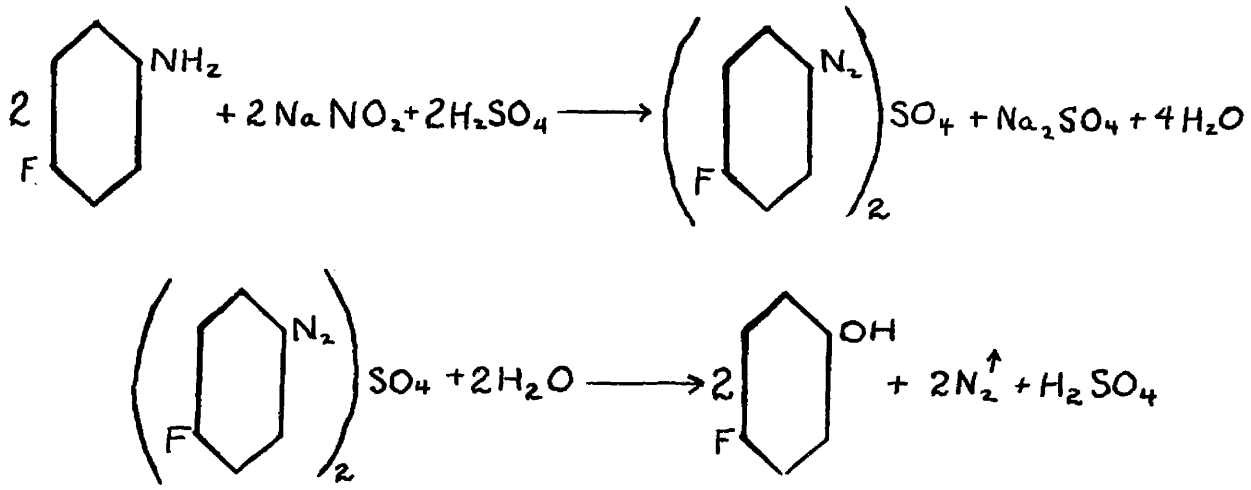
It is interesting to note that in the case of each of the three isomeric halogenated phenylmercuric chlorides, the fluoro-compound has a

[3]A sample of the compound prepared by the Grignard method melted at 245° (E. B. Starkey, unpublished). Recently Kharasch (56) reported the melting point as 243° when prepared by the Grignard method.

melting point higher than the other corresponding halogen derivatives.

XXV Preparation of p-Fluorophenol

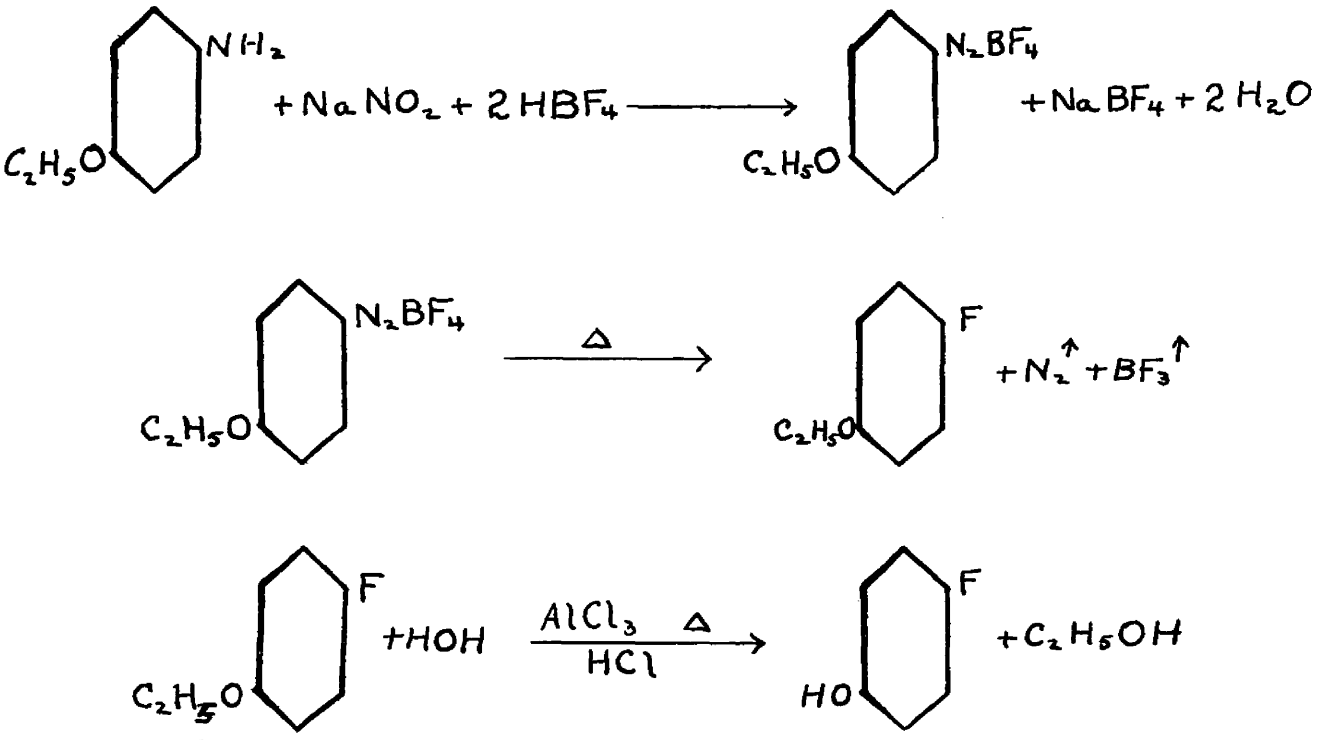
a. Preparation of p-Fluorophenol from p-Fluoroaniline



p-Fluorophenol was prepared as described by Rinkes (63) from p-fluoroaniline. The p-fluoroaniline was prepared from p-nitraniline as described in I to III. The p-fluoroaniline was diazotized according to the directions of Gatterman (64). One hundred cc. water and 20 cc. conc. sulfuric acid were mixed in a 1 l. beaker and 22 g. (0.2 mol) p-fluoroaniline stirred into the warm mixture. The solution was cooled in an ice bath and 200 g. crushed ice added, followed by a solution of 13.8 g. (0.2 mol) sodium nitrite in 60 cc. water while stirring vigorously. The cold diazonium sulfate solution was then transferred to a separatory funnel, a few pieces of ice added, and the solution allowed to drop slowly into a 2 l. round-bottomed flask containing 150 cc. of a mixture of equal parts of water and conc. sulfuric acid through which steam was passing. The steam distillate was collected. The contents of the middle flask took on a reddish color and about 1.5 g. of residue material was collected at the end of the reaction. The steam distillate was saturated with sodium chloride, shaken out with ether, the ether dried

and removed by distillation. The residue was black and on distillation under reduced pressure yielded 10 g. of yellow needles representing 40% as compared to the 75% claimed by Rinkes. Several attempts were made further to purify the product by crystallization from petroleum ether and heptane, but the crystals always retained the yellow color.

b- Preparation of p-Fluorophenol from p-Phenetidine



One-half mol or 68 g. p-phenetidine was diazotized as described under I and yielded 97 g. very pale purplish crystals[4] representing 82% of the theoretical.

The diazonium compound above was decomposed as described under XVI. The reddish distillate was washed with sodium carbonate solution and then

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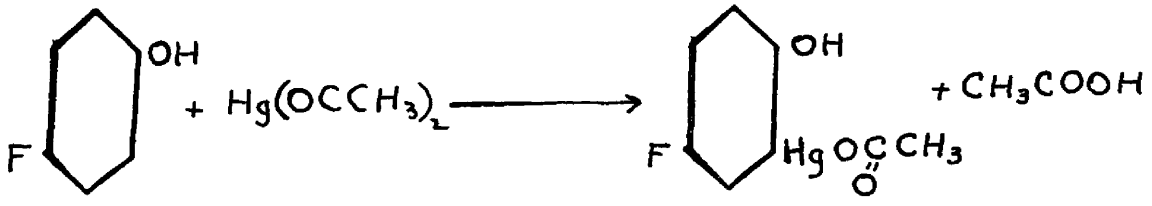
[4]The p-phenetidine was a technical grade used without purification and the diazotization mixture was highly colored. The diazonium compound darkened somewhat in color over night.

steam distilled. From the steam distillate there was separated 41 g. of very pale yellow oil resembling phenetole very closely in odor. The yield was 71% of the theoretical.

p-Fluorophenetole was converted to p-fluorophenol by the method described by Swarts (65). After sampling the p-fluorophenetole, 40 g. (0.29 mol) was placed in a 1 l. round-bottomed flask and 32 g. aluminum chloride added. The mixture was refluxed for 3 1/2 hours on an oil bath at 130-135°. With the flask immersed in a bath of cold water, an excess of 20% hydrochloric acid was added through the condenser and the mixture extracted with ether several times. The ethereal extract was then extracted with 6 portions of 20 cc. each of 10% aqueous sodium hydroxide. The combined sodium hydroxide extractives were washed with ether, made acid with sulfuric acid and re-extracted with ether. The ethereal solution was dried over calcium chloride and the ether removed by distillation. The residue of phenol was distilled under reduced pressure and crystallized in long white needles weighing 13 g. representing a 41% yield.

XXVI Preparation of o-Acetoxymercuri-p-fluorophenol



This compound was prepared by the direct mercuration of p-fluorophenol. A solution of 13 g. (0.116 mol) p-fluorophenol in 180 cc. water was treated with a solution of 36 g. (0.113 mol) mercuric acetate in 100 cc. water acidulated with acetic acid. The mixture was allowed to stand over night and 22.5 g. pale pink needles filtered off. On standing for four more days, the filtrate deposited an additional 11.5 g. of mercurial,

giving a yield of 76% in all (crystals A). The filtrate was treated with a solution of sodium chloride (till no more precipitate formed) and the curdy white solid filtered off (solid B). It weighed about 1 g. The crystals A (monomercurial) were incompletely soluble in hot alcohol, a relatively large amount of white gelatinous material appearing. The alcoholic filtrate on concentration, gave a few crystals. The monomercurial was also incompletely soluble in acetone. The crystals (A) were slowly soluble in boiling water, an amorphous solid settling out on cooling. The clear filtrate on standing several hours deposited small needles. The compound is only slightly soluble in benzene. About 20 g. of the monomercurial dissolved in 25 cc. boiling glacial acetic acid and crystallized as a dense mass of needles on cooling. The compound, on recrystallization from glacial acetic acid, became translucent at 190° and melted with decomposition 193-194°.

Solid (B) was boiled with 75 cc. alcohol and filtered. The pale yellow needle-like crystalls were redissolved in alcohol and allowed to crystallize. There remained 0.42 g. of material.

Samples (A) 0.2564, 2212 g.; HgS 0.1574, 0.1362 g.

Mercury found 52.93%, 53.10%

Calculated for $C_8 H_7 O_3 FHg$ 54.12% Hg

Sample (B) 0.2027 g.; HgS 0.1540 g.; Mercury found 65.60%

Calculated for $C_6H_4OClFHg$ 57.80%; for $C_6H_3OCl_2FHg_2$ 68.92%

On the basis of these analyses, the first and most abundant crystals were the monomercurated derivative. The small amount of solid precipitated by sodium chloride was apparently dimercurial, contaminated perhaps with a small amount of monomercurial. Since only a small amount of the

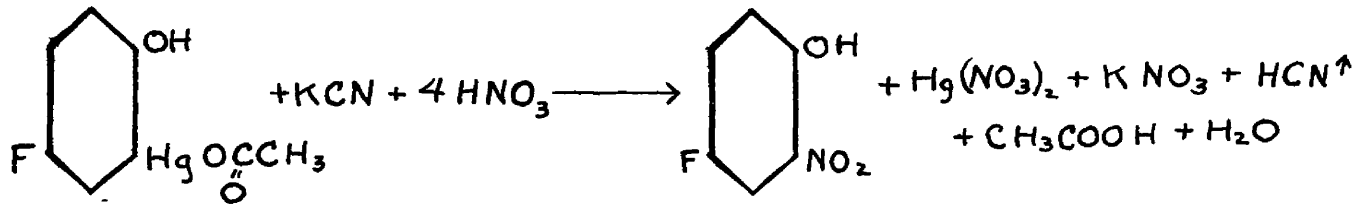
former was formed, it was not investigated further.

Some of the monomercuriacetate was dissolved in acetic acid, diluted with water and sodium chloride added. The white precipitate was filtered off and dried. This solid was dissolved in hot alcohol and filtered. On standing some nearly white needles formed. On heating these crystals very slowly, they became noticeably brown at 255°, gradually darkening in color till at 275° they turned to a dark gray, with no further change occurring up to 300°. The chloride apparently slowly decomposed.

The assumption that the mercury has entered the ring in the 2 position, that is, ortho to the phenolic hydroxyl, is based upon the following analogies. The mercuration of phenol itself leads to the formation of a mixture of the 2-mercurated, the 4-mercurated and the 2,4-dimercurated phenol (43). Since the 4 position is blocked by fluorine, a monomercurated compound would be expected to have the mercury in the 2 position in as much as the directing influence of the halogens is much less than that of the phenolic group. In addition, the facts that p-fluorophenol on nitration yielded 4-fluoro-2-nitrophenol (65), that p-fluorophenetole on nitration yielded 4-fluoro-2-nitrophenetole (65), that p-chlorophenol when heated with $HgSO_4$ is said to yield 2-mercurisulfate-4-chlorophenol (66), and that p-fluorophenol is alkylated ortho to the phenolic group (4) would seem to point to the 2 position as the location of the acetoxymercuri-group in the above described acetoxymercuri-p-fluorophenol. Hart and Anderson (67) reported the preparation of an acetoxymercuri-p-chlorophenol from p-chlorophenol and mercuric acetate. No reaction conditions were given, nor was the position of the mercury proven.

Several attempts to obtain direct evidence of the position of the mercury in the monomercurated derivative failed. Rupp (68) has described

a reaction for replacing the mercury of the two monomercurated salicylic acids with the nitro group. This reaction was applied to the mercurated



phenol in the hope of obtaining the nitrofluorophenol melting at 73.5° described by Swarts (65). Seventy two centigrams of the acetoxymercuri-p-fluorophenol was treated with 0.13 g. potassium cyanide in 10 cc. boiling water in a water bath under a hood. To this was added 16 cc. conc. nitric acid. At the instant the nitric acid was added, the solution turned a reddish brown. The liquid was heated for 10 minutes and then cooled. No crystals settled out. On standing in a refrigerator for 4 hours, no crystals appeared. The aqueous solution was extracted with ether and the ether evaporated. A mixture of reddish crystals and some thin white rectangular plates was obtained. The mixture melted at about 95°, the white crystals at 97°. This procedure was modified several times and applied to both the acetoxy- and the chloro-mercurial with no success.

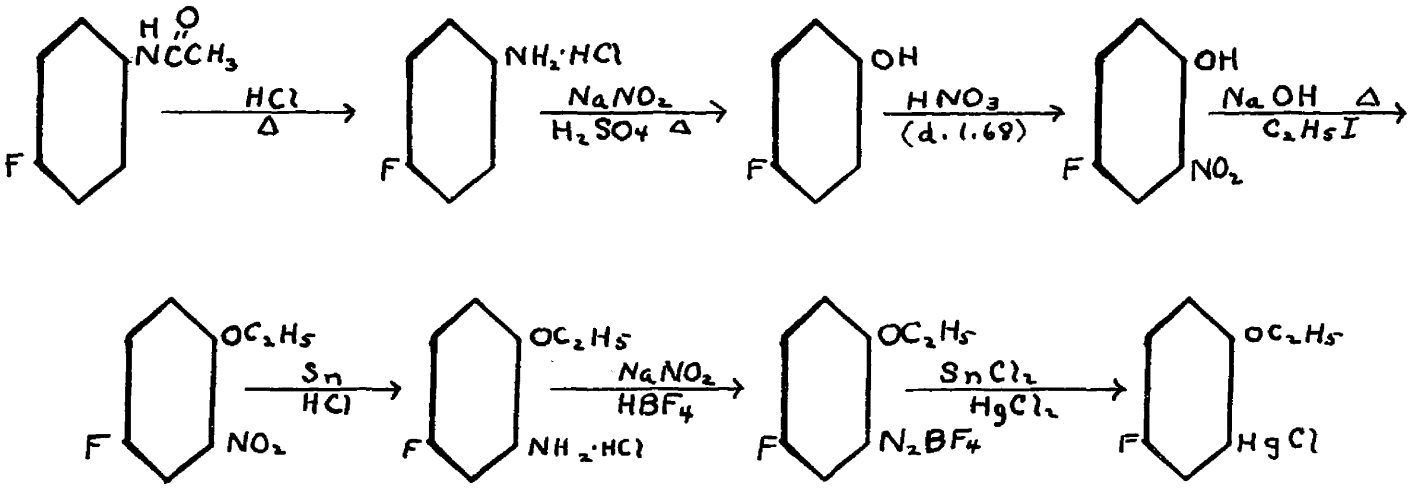
Hanke (58) reported the replacement of mercury by the nitro group in the various mercury compounds of the phenyl halides, using 68% nitric acid. One g. of the mercurial in question was mixed with 5 cc. conc. nitric acid and kept at 65° for 10 minutes. The nitro compound was precipitated with water and recrystallized. Hanke further reported that those compounds having mercury meta to the halogen did not give the reaction under the above conditions or modifications of them. When the reaction was tried on a sample of 4-fluoro-2-acetoxymercuriphenol, no

nitro compound could be isolated.

XXVII Attempts to Prove the Position of the Mercury in the Mercurated p-Fluorophenol

a- Using the Ethyl Ether

In an effort to obtain some definite substantiation of the proposed formula, the following reactions were carried out:



The liberation of the free amine from the acetyl derivative was carried out as described in XXII. From 18 g. p-fluoroacetanilid, there was obtained 15 g. of the amine hydrochloride representing 87% of the theoretical. The amine so obtained was diaztotized as described in I and converted to the phenol as described in XXVa. The yield of phenol was 4 g. or 35% of the theoretical.

The nitration was carried out as described by Swarts (65). The 4 g. of p-fluorophenol was warmed to 35° in 20 cc. water and 3 cc. conc. nitric acid added. The liquid clouded and warmed. It was allowed to cool and extracted with ether. The ether was evaporated and the residue steam distilled, yielding 3 g. (55% of the theoretical) yellow needles melting at 72°. The material was not further purified. (Swarts gave a m.p. of 73.7°).

Considerable difficulty was experienced in ethylating the phenolic group. The reaction proceeded very slowly. To a solution of 1 g. (0.025 mol) sodium hydroxide in 10 cc. alcohol was added 3 g. (0.019 mol) of nitrofluorophenol. An excess of ethyl iodide was added to the deep red solution, then 75 cc. water, and the mixture refluxed for 5 hrs. The mixture was distilled, the excess ethyl iodide boiling off first, followed by some alcohol. The remaining mixture was steam distilled. On cooling the distillate, a mass of yellow crystals melting 33.5° (Swarts (65) reported 33.7°) was obtained. A red liquid remained behind in the steam flask. This was made acid and further steam distilled. There were obtained yellow crystals melting 72-73° indicating unchanged phenol. The recovered nitro phenol was dissolved in alcoholic potassium hydroxide and boiled with excess ethyl sulfate, more potassium hydroxide or ethyl sulfate being added as required. When the addition of potassium hydroxide no longer produced a red color, most of the alcohol was removed by distillation, the material diluted with water and steam distilled. A total of 3 g. o-nitro-p-fluorphenetole was obtained, representing 85% of the theoretical.

The entire amount of nitrophenetole above was reduced with tin and hydrochloric acid as described in III to yield 1.5 g. of o-amino-p-fluorophenetole, or 60% of the theoretical.

All of amine obtained above was diazotized as described in I to give nearly white platelets.. Although this was probably a new compound, the amount of material was small and no constants were determined.

The introduction of the -HgCl group was attempted as described under VI. The acetone solution gave no mercurial. This was not repeated because of a lack of material.

b. Using the Benzyl Ether

The same series of reactions was attempted, using instead, the benzyl ether of the fluoro-nitrophenol. It was prepared as follows:

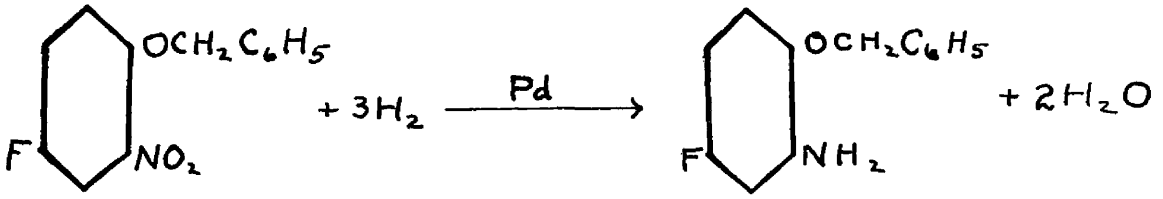
In a 500 cc. round-bottomed flask under a reflux condenser, 16 g. (0.1 mol) o-nitro-p-fluorophenol in 100 cc. alcohol was mixed with 46 cc. (0.1 mol) of a solution of sodium ethoxide in alcohol (5.2 g. sodium/100 cc. alcohol) and 14 g. (0.11 mol - an excess) of benzyl chloride. The mixture was refluxed for 5 hours. The alcohol was distilled off on a water bath and the residue steam distilled till no more yellow needles came over. The distillate was cooled and the crystals filtered off. The needles melted 70-72° indicating unchanged phenol. A brown oily layer remained behind in the middle flask. The mixture was transferred to a separatory funnel and the oil drawn off. It solidified at room temperature. It was soluble in benzene but would not crystallize. After removal of the benzene, it was dissolved in ether and precipitated as crystals by petroleum ether. These were filtered off. On evaporation of the ether-petroleum ether solution, there remained large flat rectangular plates. These were recrystallized from ether-petroleum ether to give yellow plates melting at 52°. A Kjeldahl determination as modified for nitro compounds was run.

0.8220 g. sample required 30.05 cc. 0.1047 N acid

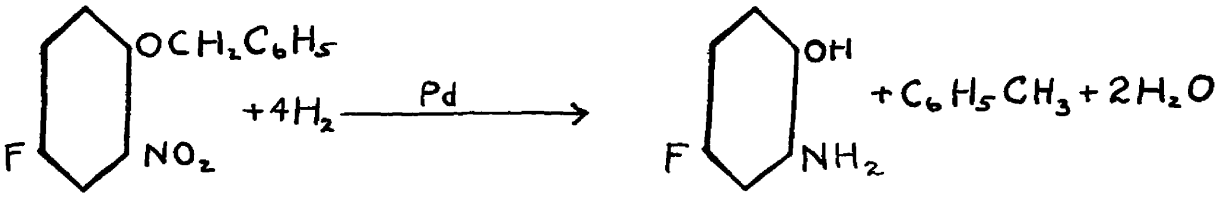
Nitrogen found 5.00%; Calculated for $C_{13}H_{10}O_3FN$ 5.66% N

Since the amount of benzyl ether was rather limited, it was decided to reduce the compound catalytically as described under III. The benzyl ether was dissolved in 75 cc. 95% alcohol, the palladium catalyst added and the hydrogenation begun. A total of 2.65 l. of hydrogen over water at 24° and 772 mm. was used or about 2.40 l. at N.T.P. while the

theoretical for the 3 equivalents of hydrogen necessary to reduce the nitro group was 1.61 l., according to the equation:



The fact that slightly more than 4 equivalents of hydrogen were consumed suggested that either the fluorine or the benzyl group had been reduced off. When the flask was opened, the odor of toluene was distinctly noticeable. The mixture was acidified with 5 cc. conc. HCl and filtered. The alcohol was removed from the mixture by distillation on a water bath under reduced pressure. The residual liquid rapidly darkened in color. The compound was obviously 4-fluoro-2-aminophenol. The reaction is shown below:



The concentrated acidic solution was neutralized with NaOH, the phenol extracted with ether, and treated with acetyl chloride. The resulting dark purplish solid was several times recrystallized from water after boiling with charcoal, and finally was obtained as a pale yellow solid. The aqueous solutions of the acetyl derivative darkened rapidly, especially when warm. The solid gave a positive test for fluorine (60). The solid melted with decomposition at 175 to 176°.

A sample of o-acetylaminophenol was prepared from o-aminophenol and acetyl chloride and purified by several recrystallizations from boiling

water and charcoal. The crystals melted 201.5° with decomposition. The melting point reported in the literature varies from 201 to 209° depending on the investigator: 201° (69, 70); 202-203° (71); 202.5-203.5° (72); and 209° (73). A mixed melting point with the above derivative showed darkening at 171° and melting with decomposition at 173°.

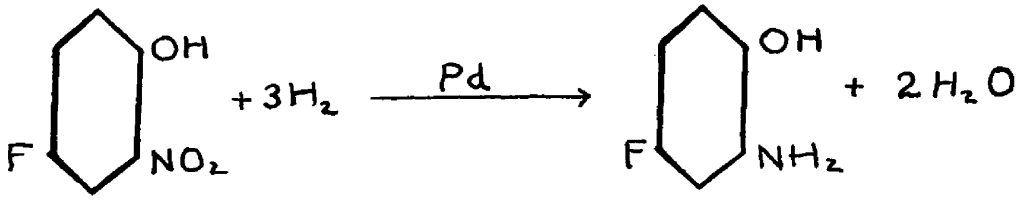
The determination of nitrogen in 4-fluoro-2-acetylaminophenol by the micro-Kjeldahl method (74) gave the following results:

Samples 5.077, 4.859, and 3.790 mg. required 2.92, 2.74 and 2.14 cc. 0.01 N HCl

% Nitrogen found 8.06, 7.90 and 7.91, average 7.96%

Nitrogen calculated for $C_8H_8O_2FN$ 8.29%; for $C_8H_9O_2N$ 9.34%

Most of the 4-fluoro-2-aminophenol from the reduction reaction was lost in the above manipulations. An attempt was made to complete the projected series of reactions using the 6 g. of 4-fluoro-2-nitrophenol which had been recovered from the benzylation reaction. This was reduced with palladium catalyst and hydrogen as described in III.



The sample required 2.73 l. hydrogen at 26° and 770 mm. (over water). On conversion to N.T.P., 2.60 l. hydrogen was used compared to 2.56 l. theoretical. The reaction mixture was treated with 5 cc. HCl, filtered to remove the charcoal and then concentrated under reduced pressure on a water bath while passing in a stream of carbon dioxide.

The light brown residue from above was dissolved in 10 cc. water,

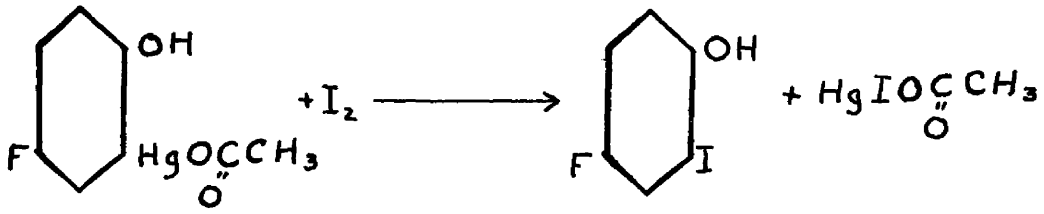
added to 15 cc. HBF_4 and diazotized in an ice salt bath, maintaining the temperature at 0°. When the addition of sodium nitrite was complete, no solid settled out, nor could any be precipitated with alcohol and ether. The diazonium borofluoride was quite soluble. In this connection, it has been pointed out (75) that the o- and p-hydroxyphenyl diazonium borofluorides are quite soluble in water.

To the above purplish red solution was added 20 g. $HgCl_2$ and 1 g. charcoal with stirring in the ice bath. To this mixture was slowly added 18 g. $SnCl_2 \cdot 2H_2O$. The diazonium compound decomposed. At the end of the reaction, the mixture was centrifuged and the water decanted. The solid was washed twice with water, the combined water being wine red. No mercurial could be separated from the aqueous extract so it was discarded. The solid was then washed with warm acetone. The brown acetone solution on adding water gave a brown precipitate which contained no mercury and was discarded. The reaction solid was then extracted with dilute sodium hydroxide and filtered. The red solution was boiled with charcoal and the dark amber filtrate acidified with acetic acid. The brownish precipitate was filtered off. It contained mercury. This solid was extracted with boiling glacial acetic acid in which 4-fluoro-2-acetoxymercuriphenol had been found to be very soluble. Most of the solid remained insoluble. The reddish filtrate was concentrated and when nearly dry left some long whitish needles which melted about 95-100°. Nothing having the properties of the desired mercurial could be separated.

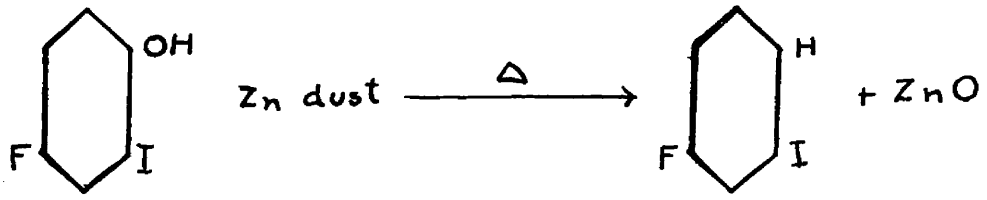
c- Replacement of Mercury with Iodine

Mercury may easily be replaced by the halogens iodine (76) and bromine. However, the corresponding halogenated fluorophenols have not

been reported. An effort at determining the position of the mercury was made by replacing the mercury with iodine in the hope that it would be possible to convert the iodo-derivative to some known compound.

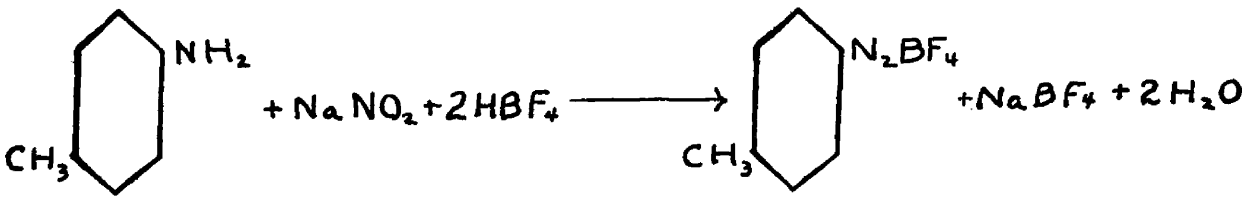


To 7.4 g. of the mercurial (0.02 mol) in 20 cc. chloroform in a 200 cc. round-bottomed flask was slowly added with stirring 5.1 g. (0.02 mol) iodine. The mixture warmed some. After one hour the mercuric iodide was filtered out and the chloroform filtrate washed with aqueous potassium iodide solution to remove any free iodine. The chloroform was removed on a water bath and an attempt made to distill the residual thick liquid under reduced pressure. The formation of free iodine vapors was noted and the distillation stopped. The residue in the flask was dissolved in dilute sodium hydroxide, acidified and extracted with chloroform. The chloroform was removed on a water bath. The residue was a very thick reddish brown oil having a distinctly phenolic odor and giving a purplish color with ferric chloride. It did not crystallize on chilling in a refrigerator. No further purification was attempted. Some of the compound was transferred to a small distilling flask with ether and the ether removed. A few pellets of sodium hydroxide were added and the mixture heated. A few drops of yellowish liquid distilled up on the sides of the flask. This material gave a violet color with ferric chloride which was discharged by sodium acetate (a color reaction of resorcinols). This is not, however, very significant since either o-iodophenol or p-chlorophenol on alkali fusion give resorcinol.



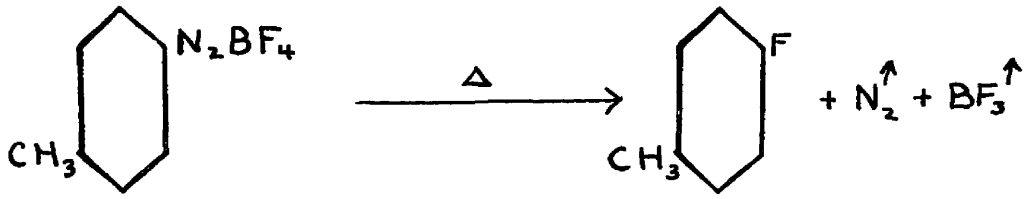
Since m-iodofluorobenzene is known (28) and is a liquid having a characteristic refractive index, it was thought that it might be possible to remove the hydroxyl group with zinc dust. A small sample of the phenol was transferred to a flask with ether and the ether removed. The equivalent amount of zinc dust was added and the mixture first warmed on a water bath. Apparently no reaction occurred. The flask was then heated with a direct flame. A few droplets of liquid giving a purplish color with ferric chloride distilled up on the neck of the flask but no liquid having the properties of m-iodofluorobenzene could be found. The residue in the flask was extracted with alcohol which on evaporation left a black residue. It was thought possible that some coupling might occur to give m,m'-difluorodiphenyl which is a liquid (20) boiling at 130° at 14 mm. However, its formation could not be shown either.

XXVIII Preparation of p-Methylphenyldiazonium Borofluoride



One half mol, 53.5 g., p-toluidine was diazotized in the manner described in I to give 85 g. of nearly white diazonium compound representing 83% of the theoretical. This yield was duplicated in subsequent trials.

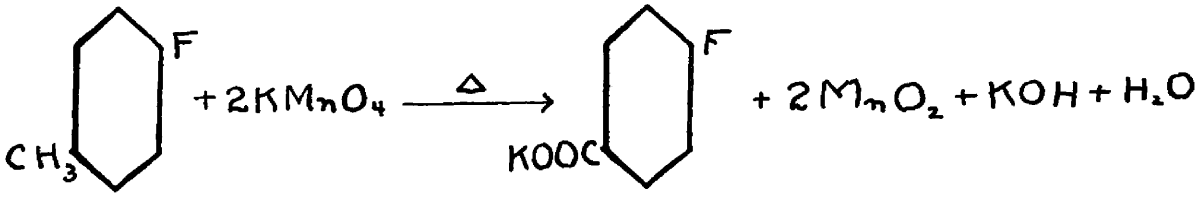
XXIX Preparation of p-Fluorotoluene



The above diazonium compound was decomposed as described under XVI to give 21 g. of colorless oil resembling toluene in odor (46% yield). In subsequent experiments the yield was raised to 52% and 69%. Schiemann reported 97% (11).

XXX Preparation of p-Fluorobenzoic Acid

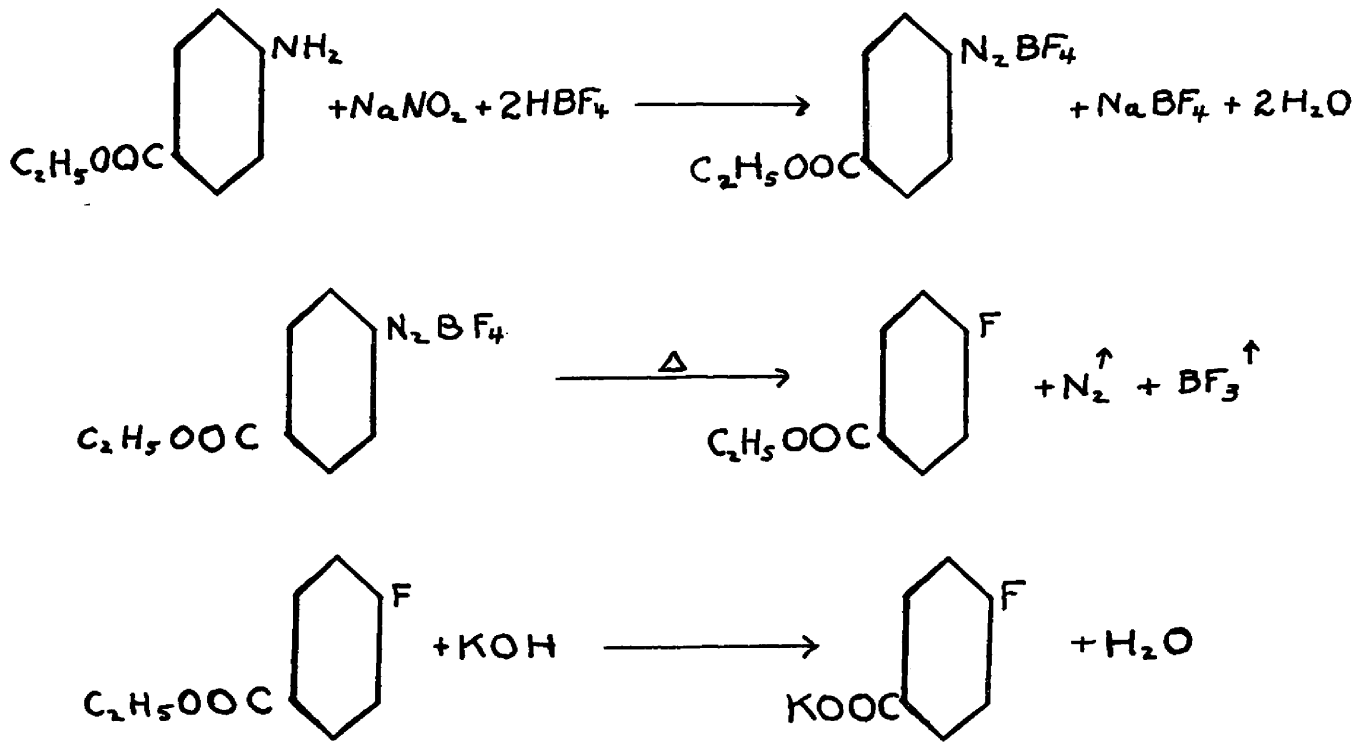
The oxidation of p-fluorotoluene using chromic acid in acetic and sulfuric acids (77) at room temperature was too vigorous and resulted in tar. The reaction was repeated, cooling the flask in an ice bath, but only about 0.5 g. p-fluorobenzoic acid was obtained from 10 g. p-fluorotoluene. An attempted oxidation using potassium permanganate in glacial acetic acid along with a little sulfuric acid also led to tar.



The oxidation was carried out in aqueous permanganate (78) as follows: 10.5 g. (0.096 mol) p-fluorotoluene, 10.5 g. potassium permanganate and 250 cc. water were refluxed for 6 hours, more permanganate being added as required. The hot mixture was then filtered and concentrated. The solution was acidified with hydrochloric acid and the white solid filtered off. The solid was crystallized from boiling water to give 5 g. white needles melting 183-184° (Schiemann (79) gave the m.p. as

186°), representing 38% of the theoretical. In subsequent experiments, yields of 52, 58, and 53% of the theoretical were obtained.

Additional p-fluorobenzoic acid was also prepared as outlined by Schiemann and Winkelmüller (79).

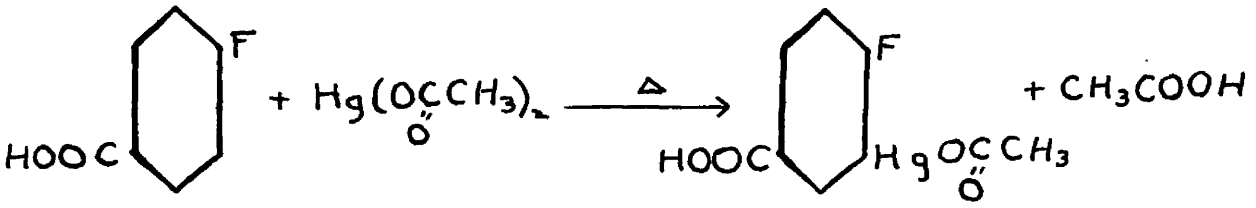


About one-half mol (81 g.) ethyl-p-aminobenzoate was diazotized in 200 cc. borofluoric acid as described under I to give a quantitative yield or 132 g. of diazonium compound.

All of the diazonium compound was packed in a 2 l. distilling flask, the delivery arm of which was connected to a 1 l. distilling flask cooled under tap water. Its delivery arm was connected to a water pump through a trap containing sodium hydroxide. The solid was slowly decomposed beginning at the edges, finally heating strongly. The solid was extracted with ether, the ether distilled off, and the residue boiled under a reflux condenser with 30 g. KOH in 40 cc. 95% alcohol and 60 cc. water for 1 1/2 hours. The solution was filtered hot

and then made acid to congo red with conc. HCl. The precipitated acid was filtered off, recrystallized twice from water and melted 180°. A total of 34 g. or 50% of the theoretical was obtained.

XXXI Mercuration of p-fluorobenzoic acid



In a 500 cc. round bottomed flask under a reflux condenser, 6 g. (0.043 mol) p-fluorobenzoic acid and 13.7 g. (0.043 mol) mercuric acetate were heated in 50 cc. glacial acetic acid for 6 1/2 hours in a test run. At the end of this time, a sample of the solution no longer gave a yellow precipitate when made alkaline with sodium hydroxide. The mixture was cooled and 2.5 g. of white solid filtered off. Attempted recovery of more mercurated product by concentration of the filtrate, led to decomposition of the compound with the liberation of p-fluorobenzoic acid. The properties of the sample filtered off at the end of the reaction were studied. The solid was dissolved in dilute sodium hydroxide. A small amount of black solid remained and was filtered off. Evidently the product was present partly as the mercuric salt. The filtered alkaline solution was cautiously acidified with dilute hydrochloric acid while cooling the mixture. The white solid was filtered off, dried and dissolved in hot alcohol. The solid came out in an amorphous state on cooling. Solutions of the compound in acetone also settled out amorphous material. The mercury was rather easily split out by boiling the solution with hydrochloric acid. The compound was completely soluble in

sodium hydroxide without giving either a black or yellow precipitate, was precipitated from alkaline solutions by acids, gave a positive test for fluorine by the Feigl test (60) and for mercury after treatment with hydrochloric acid or bromine. A sample was analyzed with the following result:

Sample 0.1884 g.; HgS 0.1166 g.; Mercury found 53.36%

Calculated for $C_6H_4O_2ClFHg$ 53.50% Hg.

The above reaction was repeated with somewhat similar results. The yield was poor. Twenty g. of p-fluorobenzoic acid when treated as above yielded 10.5 g. of what was thought to be the chloromercuri compound. The compound was dissolved in dilute sodium hydroxide and 2.5 g. of black mercuric oxide filtered out. The clear filtrate was carefully acidified with hydrochloric acid, the white solid filtered off, washed well with water and dried. The solid was dissolved in hot alcohol and allowed to settle out on cooling. An analysis of this material gave 46.08% mercury as compared to 53.50% required by theory. The alcoholic mother liquor above settled out some additional material on concentration, which after recrystallization gave the following analytical data:

Samples 0.1900, 0.1898 g.; HgS 0.0235, 0.0233 g.

Mercury found 10.66, 10.58%

A sample of this material softened at 165° and melted 170-175°. It was probably a mixture of p-fluorobenzoic acid and some of the mercurated compound.

At some point in the manipulations described, the mercury has been split off to regenerate p-fluorobenzoic acid. The total powder remaining from the experiment weighed 10 g. and was extracted with ether. The insoluble residue weighed 4 g. The ethereal extract on concentration

deposited white needles which melted 178-180° without further purification. This material is probably p-fluorobenzoic acid. The insoluble material above was shown to contain mercury and fluorine, to be soluble in alkali and insoluble in acid. It was dissolved in hot alcohol, and the white amorphous powder formed on cooling was filtered off and dried in a desiccator. The analyses gave the following:

Samples 0.2165, 0.1915; HgS 0.1382, 0.1236; Mercury found 55.03, 55.12%

Calculated for $C_6H_4O_2ClFHg$ 53.50 % Hg

The compound when heated in a melting point tube slowly turned yellow above 230° and melted with dec. at 239°.

Several attempts were made to locate the position of the mercury by replacement with the nitro group according to the reaction described by Rupp (68) and modifications of it. When the reaction was carried out as described in the literature, using a mixture of equal parts nitric acid and water, a white solid crystallized from the solution on cooling. It still contained some mercury and seemed to be mostly unchanged mercurial. The test was repeated using instead, a mixture of 1 part water and 2 parts nitric acid. On cooling white needles which melted gradually between 170 and 180° separated[5]. Further concentration of the filtrate separated white needles which melted at 172-176° and which were apparently p-fluorobenzoic acid. Another trial gave long white needles which melted at 175°. None of these crystals were purified because of the small amounts available but the assumption

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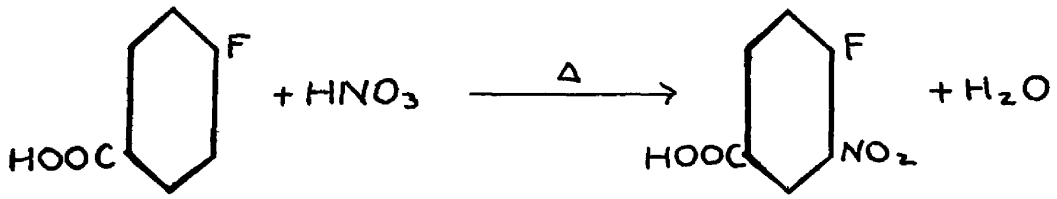
[5]The two possible nitrofluorobenzoic acids are 3-nitro-4-fluorobenzoic acid melting 121-122° (80) and 2-nitro-4-fluorobenzoic acid melting 130° (81).

that they represent impure p-fluorobenzoic acid seems justified. They are obviously neither of the two mononitrated p-fluorobenzoic acids.

Since the results obtained on the structure of the mercurated benzoic acid were not very conclusive, attempts were made to prepare the same compound by replacing the amino group of 3-amino-p-fluorobenzoic acid with mercury. The following reactions were then carried out.

XXXII Preparation of 3-Nitro-4fluoro-benzoic Acid



The method is that given by Slothouwer (80). To 50 g. (33 cc.) nitric acid (d. 1.52) heated to boiling was added 7. g. (0.05 mol) p-fluorobenzoic acid. It dissolved rapidly. After all the material had been added, the mixture was heated gently for 1/2 hour and then poured into a beaker containing crushed ice. The solid was filtered out with suction. The filtrate smelled strongly of p-fluoronitrobenzene, the product of a side reaction. Only 3 g. of crystals melting at about 100° were obtained.

The nitration of another sample of p-fluorobenzoic acid was attempted as above but the time of heating was shortened in an effort to reduce the loss of material by conversion into p-fluoronitrobenzene. When the mixture was poured onto ice, filtered and washed, a sample of the solid melted 160-165° indicating impure, unchanged p-fluorobenzoic acid.

The nitration was then attempted on the p-fluorobenzoic acid re-

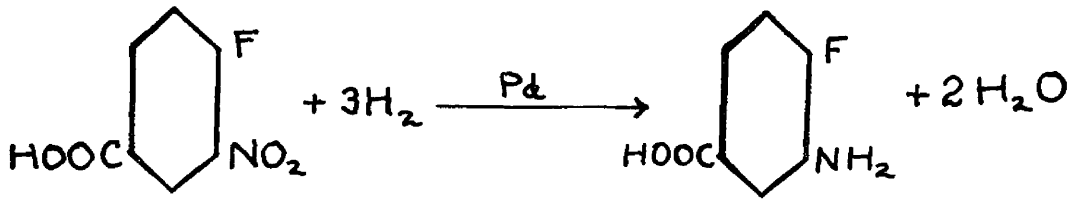
covered above by the method described by Rouche (82). To 150 cc. cold nitric acid (d. 1.52) was added all of the p-fluorbenzoic acid recovered and the mixture heated for 2 1/2 hours on a water bath under a reflux condenser. The mixture was poured onto ice and allowed to stand in the refrigerator over night. The solid was filtered off and melted 160-165°. The filtrate was concentrated and separated a little pale yellow granular material which softened 110°, melted 115° and gave a positive test for nitro group (83).

The nitration of a new lot of p-fluorobenzoic acid was carried out by method of Rouche using fuming nitric acid recently obtained. A yield of 14.5 g. of nitrated product was obtained from 22 g. of p-fluorobenzoic acid, representing 50% of the theoretical. Apparently the fuming nitric acid used in the first three experiments had deteriorated in some way.

p-Fluorobenzoic acid was also nitrated with a nitrating mixture of sulfuric and nitric acids. To a mixture of 48 cc. concentrated sulfuric acid and 45 cc. concentrated nitric acid was added 14 g. p-fluorobenzoic acid and the mixture heated on a water bath under a condenser for 2 hours. The mixture was then poured onto cracked ice and filtered. A small amount of solid was obtained. The filtrate was concentrated and partially neutralized with sodium carbonate. On standing, crystals formed which were extracted with ether. The solid obtained by the evaporation of the ether gave a positive nitro test (83). All of the nitrated product was combined, dissolved in sodium carbonate and extracted with ether to remove any p-fluoronitrobenzene. The alkaline solution was then neutralized with acid, extracted with ether and the ether evaporated. The solid was crystallized several times from boiling

water, giving nearly white needles melting 121.5 to 122°.

XXXIII Preparation of 4-Fluoro-3-aminobenzoic Acid



The nitro group was reduced using hydrogen and palladium catalyst. A solution of 13.5 g. (0.073 mol) of 4-fluoro-2-nitrobenzoic acid in 90 cc. 95% ethyl alcohol was reduced as described in III. The hydrogen consumed measured 5.37 l. at 24° and 812 mm. over water which is equivalent to 5.17 l. (0.237 mol) at N. T. P. compared to the theoretical of 4.91 l. (0.219 mol). The reaction mixture was filtered and the alcohol removed by distillation on a water bath at reduced pressure. A total of 11 g. brown needles was obtained corresponding to 98% of the theoretical.

A small sample of the free amine was treated with acetyl chloride, the excess of the latter decomposed with water and the solid boiled with water and charcoal. The pale yellow filtrate on concentration yielded tan needles. The crystals were further purified by repeating the treatment with charcoal in water. The crystals did not show a sharp melting point, decomposing from 200° up, finally liquefying from 230 to 240° depending on how long the solid was kept above 200°. If however, the capillary was immersed in the bath at 240° and the temperature very slowly raised, it melted with decomposition 245 to 246°.

Another sample of the amine was treated with an excess of HCl and boiled with charcoal several times. The final solution was concentrated in a desiccator over $CaCl_2$ and yielded small white plates, which began

to darken at 215° and melted with decomposition 240 to 243°.

A sample of amine was crystallized several times from boiling water and charcoal and was obtained as light brown needles (the nearly colorless solution darkened rapidly white filtering). These crystals melted with decomposition at 176°. A sample of 4-fluoro-3-aminobenzoic acid obtained in another experiment melted with decomposition at 182 to 183°.

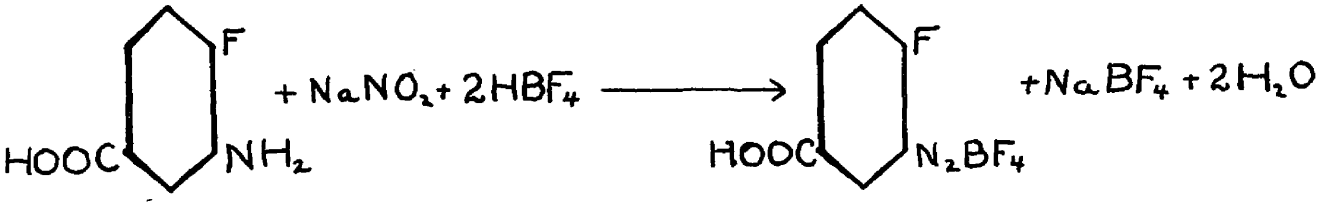
A sample of the purified acetyl derivative was analyzed for nitrogen by the micro-Kjeldahl method (74).

Samples 5.110, 4.012, and 4.859 mg. required 2.61, 2.07 and 2.44 cc. 0.01 N HCl

% Nitrogen found 7.16, 7.23, 7.03; average 7.14%

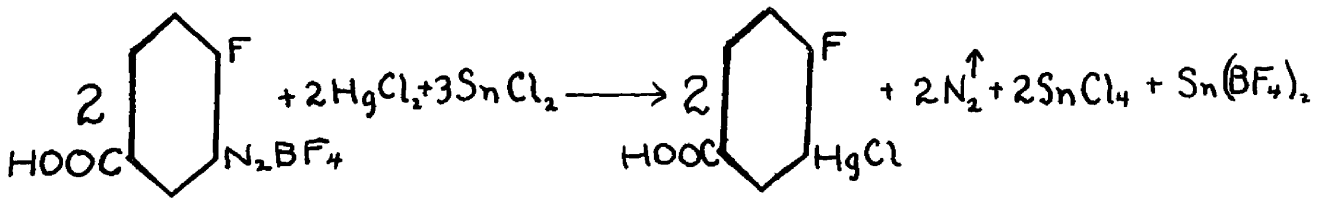
Calculated for $C_9H_8O_3NF$ 7.11% Nitrogen

XXXIV Diazotization of 4-Fluoro-3-aminobenzoic Acid



The 8 g. of amine available from XXXIII was added with stirring to 21 cc. borofluoric acid and diazotized as described in I. There was obtained 10 g. of cream-colored powder representing 77% of the theoretical. The compound darkened at 175° and decomposed at 185°.

XXXV Preparation of 4-Fluoro-3-chloromercuribenzoic Acid



The replacement of the amino group with mercury by the method described under VI gave a very poor yield. However, from the experiment outlined below, about 1 g. of colored mercurial was obtained. It was very difficult to purify. To a cooled and stirred mixture of 11.0 g. (0.048 mol) stannous chloride $\cdot 2H_2O$ and 6.5 g. (0.024 mol) mercuric chloride in 25 cc. acetone and 10 cc. water was added a suspension of 6.0 g. (0.024 mol) 4-fluorobenzoic acid-3-diazoniumborofluoride and 9.0 g. (0.033 mol) mercuric chloride in 75 cc. acetone. When the reaction was complete as shown by the absence of a red color when a few drops of the reaction mixture were tested with an alkaline alcoholic solution of B-naphthol, the mixture was made alkaline with 28% ammonium hydroxide and filtered through a Buchner funnel. The solid was washed on the funnel several times with water and the combined filtrate and washings (orange) then acidified with hydrochloric acid. The orange precipitate was filtered off on a Buchner funnel and washed with water. The purification of the compound by boiling the alkaline solution with charcoal, by crystallization from glacial acetic acid, acetone or benzene, or by washing with ether was unsuccessful. By continued repetition of solution in acetone and precipitation with petroleum ether, there was eventually obtained a small amount of a pale yellow amorphous solid. It melted with decomposition at 241°. A mixed melting point of this material with a sample obtained by direct mercuration gave a melting point of 239° with dec. The compound prepared by replacement of the diazonium group gave positive qualitative tests for the presence of fluorine and non-ionizable mercury. These tests would seem to indicate identity of the two compounds.

Additional evidence was obtained as a result of the following

observation. When the compound decomposed at its melting point, bubbles of gas rose through the melt and a deposit of white needles sublimed on the upper cool portion of the tube. A sample of material prepared by mercuration was heated at 240° in a piece of 7 mm. glass tubing sealed at one end until decomposition appeared to be complete. The tube was removed from the bath, cut above and below the sublimate, and the crystals scraped out. A sample of the crystals and a sample mixed with 2-fluoro-phenylmercuric chloride when heated simultaneously, melted 159° and 160° respectively. This was taken as evidence that the mercury entered the ring ortho to the fluorine in the direct mercuration. These data along with the melting point behavior of the mercurated fluorobenzoic acid prepared by the two methods described was taken as establishing the formula of the compound as 4-fluoro-3-chloromercuribenzoic acid.

PHARMACOLOGY

Literature Survey

The change in pharmacological or physiological action brought about by the introduction of the halogens chlorine, bromine and iodine into the organic molecule have been investigated extensively. The effect of the introduction of fluorine on the physiological properties of organic compounds has not yet been thoroughly studied. Swarts, in a comprehensive review (51) of the field of organic fluorine compounds in 1924 made the statement "I have not mentioned the physiological properties of organic fluorine compounds for the good reason that they have not been studied." Frankel in "Die Arzneimittel Synthese" (84) devoted less than a page to organic fluorine compounds. Schiemann in 1930 (85) said "Not much is known about the physiological properties of organic fluorine compounds." Bockemuller (7) in a review of organic fluorine compounds published in 1936 devoted 2 pages out of 100 to a general discussion of physiological properties. Such a condition may be the result of one or both of two considerations, namely the fact that the chemistry of the fluorine compounds has not been worked out as well as that of the other halogen compounds and the fact that the early studies of Tappeiner (86, 87) and Schulz (88) showed that inorganic fluoride ions exhibited an action of little therapeutic importance and were highly toxic.

The literature on the toxicity of inorganic fluorine compounds is voluminous and has been very well summarized by McClure (89). The possible effects of traces of fluoride ion in drinking water and foods has

been intensively investigated since the preliminary report of M. C. Smith, Lantz and H. V. Smith (90) suggesting the relation of the fluoride ion to the mottling of teeth. The effect of fluorides in the rations of live stock and its resultant secondary effect on human beings through the ingestion of milk, eggs etc., has been studied (91-96).

To date, the occurrence of mottled enamel as the result of the administration of organic fluorine compounds is limited to one substance. Kempf, Greenwood and Nelson (97) found that alpha-fluoronaphthalene, when fed in doses containing 0.05% F, caused mottled enamel in rats, whereas fluorobenzene, p-fluorobenzoic acid and p,p'-difluorodiphenyl had no effect on teeth in the same dosage.

As early as 1926 Goldemberg (98) showed that inorganic fluorides when given in small doses over long periods of time led to thyroid hypertrophy and suggested the possibility of a relationship between endemic goiter and cretinism and fluorine compounds. This work was continued by Goldemberg (99 to 104) and by Schteingart and Sammartino (105). Sodium fluoride has been administered in natural and experimental hyperthyroidism and in Basedow's disease with varying results (106, 107). Phillips, English and Hart (108) found that chronic fluorosis induced by feeding sodium fluoride caused non-toxic levels of desiccated thyroid to become toxic.

Purjesz et al. (95) showed that hens injected with sodium fluoride stored the fluorine in the eggs. They observed the fact that these eggs containing fluorine when fed to patients with Basedow's disease, produced a significant fall in pulse rate, body temperature and basal metasolism and an increase in body weight. This work is referred to and extended by Phillips and Hart (96). By careful separation of the portions of

the egg and by extraction with selective solvents, they found that no fluorine was stored in the shell, only traces in the albumin and the most of it in the yolk, especially in the lipoid fraction. The presence of significant amounts of fluorine in the eggs of hens being fed mineral supplements containing fluorides, presents a necessity for care, particularly in infant feeding.

Abelin (109), after studying the effect of thyroxin, tyrosine and diiodotyrosine on animals came to the conclusion that tyrosine could be used to treat animal hyperthyroidism symptomatically and that the good results ascribed by others to diiodotyrosine could be attributed to the summation of the beneficial action of both iodine and tyrosine. In 1936 Litzka (110) reported the results of an extensive series of studies on 3-fluorotyrosine[6] first prepared by Schiemann, Winkelmüller and Roselius (21). Treatment with fluorotyrosine produced an increase in body weight in animals suffering from an experimental hyperthyreosis. Fluorotyrosine opposed the glycogen depletion of the organs brought about by doses of thyroxin or of the thyreotropic hormone of the anterior pituitary. In clinically sound persons, the administration of large doses of fluorotyrosine produced a fall in the blood sugar level. While severe and moderately severe diabetes will probably always remain in the domain of insulin therapy, it is possible that in light cases, the carbohydrate balance may be improved by doses of fluorotyrosine thereby conserving insulin (111).

Litzka (110) pointed out the rather surprising fact that the m.l.d.

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[6]Fluorotyrosine is covered by the German Patent 621,862 (1932) and is to be sold under the name "Pardinon" by Bayer, I. G. Farbenindustrie, A. G., Leverkusen.

of fluorine in fluorotyrosine was 1/18th of that for sodium fluoride for white mice and about 1/200th of the dose for guinea pigs. However, when given in the proper dosage, fluorotyrosine exhibited no cumulative action and, on chronic administration, did not lead to the severe organic disturbances observed with sodium fluoride. When 10 persons were given as much as 6 mg. of fluorotyrosine daily, the urine showed no albumin, sugar, urobilinogen or sediment. In clinically sound persons with a tendency toward hypertension, there was a slight fall in blood pressure noticeable in 2 to 4 hours (112). Fluorotyrosine acted by partially or totally paralyzing the metabolic action of the thyroid gland secretions circulating in the body (113).

Kraft (114) in studies on metamorphosing tadpoles showed that less fluorotyrosine was required to antagonize the effect of a given dose of thyroxin than of either sodium fluoride or o-fluorobenzoic acid. He pointed out the fact that fluorine exists in significant amounts in the blood and that the accepted value for the ratio of blood iodine to blood fluorine based on the average analyses of many authors is 1 to 7. In tadpoles, the active ratio of thyroxin to fluorotyrosine was such that when calculated on the basis of iodine to fluorine, the ratio was 1 to 7. From these facts he made the interesting speculation that the "antithyroidal protective" postulated in the blood may be a fluorine containing substance, perhaps quite similar to fluorotyrosine.

The effects of the inorganic fluorides on circulation, blood fluorine content, coagulation time and heart have been investigated (115-117). K. Lang (118) showed that the ingestion of fluorobenzene, p-fluorotoluene or p-fluorobenzoic acid produced no change in the blood fluorine content or in the coagulation time. Fluorobenzene, p-fluorobenzoic acid and p,p'-difluorodiphenyl did not cause any significant

variation in the hemoglobin content of the blood (114). Sodium fluoride when injected intravenously in dogs caused a slight fall in blood pressure (119). 3-Fluorophenylethylamine and other fluorinated ethylamines have been prepared and are claimed to have pressor activity (23). H. L. Hanson (53) reported the preparation of several 3-chloro- and 3-fluoro-derivatives of adrenalone. The halogenated derivatives were weaker in pressor activity than adrenalone itself and the fluoro-compounds were less active than the corresponding chloro-compounds. No comparison of their toxicities was made.

Lang (118) studied the effects of feeding fluorobenzene, p-fluorotoluene and p-fluoroacetanilid on rabbits over an extended period. There was no increase in blood fluorine, no change in blood chemistry and no storage of fluorine in the body with the possible exception of the heart, where the fluorine content may increase from 2 to 5 times the normal value. The metabolism of the compounds was also studied. p-Fluorotoluene was excreted as p-fluorobenzoic acid[7] but the fate of fluorobenzene and p-fluoroacetanilid could not be determined. Without giving the experimental data, he stated that the pharmacological action of the substances was similar to that of the parent compounds and gave no indication of a specific action of fluorine.

The effect of organic fluorine compounds on the central nervous system has been investigated to some extent. Lehmann (120) compared the activities and toxicities of the following 9 compounds on frogs: toluene, trichlorotoluene, trifluorotoluene, chloro-difluorotoluene,

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[7]Coppola (121) fed the three isomeric fluorobenzoic acids to dogs and claimed to isolate the corresponding hippuric acids from the urine.

m-nitro-trifluorotoluene, the hydrochlorides of aniline, m-aminotoluene, and m-aminotrifluorotoluene, and the sodium salt of m-trifluoromethylbenzoic acid. The conclusions may be summarized as follows: as the hydrogens of the side chain were replaced with halogen, the toxicity increased, fluorine being more active in this respect than chlorine. In addition, any excitatory properties the compound may have had, were depressed and the paralyzing action was increased. As evidence of the increased narcotic properties resulting from the replacement of the hydrogens of the methyl side chain by fluorine, Lehmann stated that 3 to 6 mg. of m-trifluoro-toluidine hydrochloride in aqueous solution may be used as a narcotic for frogs.

Midgely and Henne (1) and Henne (2, 3) have described the nontoxic properties of the gas dichloro-difluoromethane patented and trademarked as "Freon 12" (refrigerant). Neither "Freon 12" nor fluoroform have marked narcotic activity. Animals survive in an atmosphere in which all of the gases except oxygen have been replaced with fluoroform. The substitution of chlorine by fluorine in these compounds has decreased the toxicity. Related compounds are described in a patent (122) and by Booth and Bixby (123).

Yant (124) studied the results of the exposure of dogs and monkeys to difluorodichlormethane and dichlorotetrafluoroethane. The toxicity of these compounds is low and their effective concentrations are roughly 50 to 100 times those of chloromethane, chloroform and carbon tetrachloride. Exposure to a concentration of 20% by volume of dichlorodifluoromethane caused marked symptoms (irritation and tremors) which disappeared very rapidly after removal from the gas. Continuous exposure to this concentration for 121 hours produced marked symptoms but

no unconsciousness or death. Dichlorotetrafluoroethane was somewhat more toxic but the symptoms also disappeared rapidly after termination of exposure. Brenner (125) has studied the effects of dichlorodifluoromethane on cats, dogs and monkeys. The gas was given by inhalation or by tracheal tube along with sufficient oxygen to prevent asphyxia. In spinal animals, it produced a definite depression of the reflex responses tested; in preparations in which the section was above the level of the red nucleus, there was an increased hyperactivity of certain reflexes; in decorticate preparations, slight tremors of the extremities occurred; in normal animals marked tremors of the head, facial musculature and legs developed. The tremors produced on inhalation are probably "release" phenomena resulting from an effect of the gas on the regulatory motor centers.

Swarts made some interesting observations on the irritant properties of derivatives of toluene (51). Trifluorotoluene resembled toluene very closely in odor and, in contrast to trichlorotoluene, was quite inoffensive. In some instances, the presence of the fluorine atom considerably increased the irritant properties of compounds already containing chlorine or bromine atoms. For example, difluorochlorotoluene was distinctly more irritant on mucous membranes than chlorotoluene (51). Swarts (65) stated that 1-fluoro-2,4-dinitrobenzene when applied to the skin stained it yellow and caused a burning sensation. This was attributed to the fact that the fluorine is easily split out of the dinitrobenzenes and thus exerts its irritant action (7). The aliphatic acid fluorides are quite irritant and resemble in activity the corresponding chlorine, bromine and iodine compounds (51).

The pharmacological tests carried out with meta and para fluoro-

acetanilids will now be described. Acetanilid was used as the reference standard for antipyretic activity.

Experimental

In the present investigation, the antipyretic activities of m- and p-fluoroacetanilid and of p-chloroacetanilid and the relative toxicities of acetanilid and p-fluoroacetanilid were studied. A review of the literature showed that the laboratory tests on the toxicity of acetanilid have been carried out using most of the common laboratory animals and the tests of antipyretic activity have been performed on rabbits and white rats. All of the experiments to be described were performed on cats.

It was thought desirable to note whether or not there would be any effect on circulation and respiration when p-fluoroacetanilid was given intravenously. The carotid blood pressure and respiration (by tracheal canula) were simultaneously recorded in the usual manner during the perfusion of a solution of p-fluoroacetanilid (0.5 g. per 100 cc.) in 10% alcohol[8] into the femoral vein. The required operative procedures were carried out under ether anesthesia. After the perfusion had been begun, it was usually possible to remove the ether cone entirely for the remainder of the experiment. The solution was administered at a rate sufficient to cause the death of the animal in from 2 1/2 to 3 1/2 hours. Controls were run using a solution of acetanilid of the same strength and a solution of 10% alcohol alone. Figure 1 (p. 59) shows two sections of the tracings, the first taken at the beginning of the experiment and the second after a period of 65 minutes.

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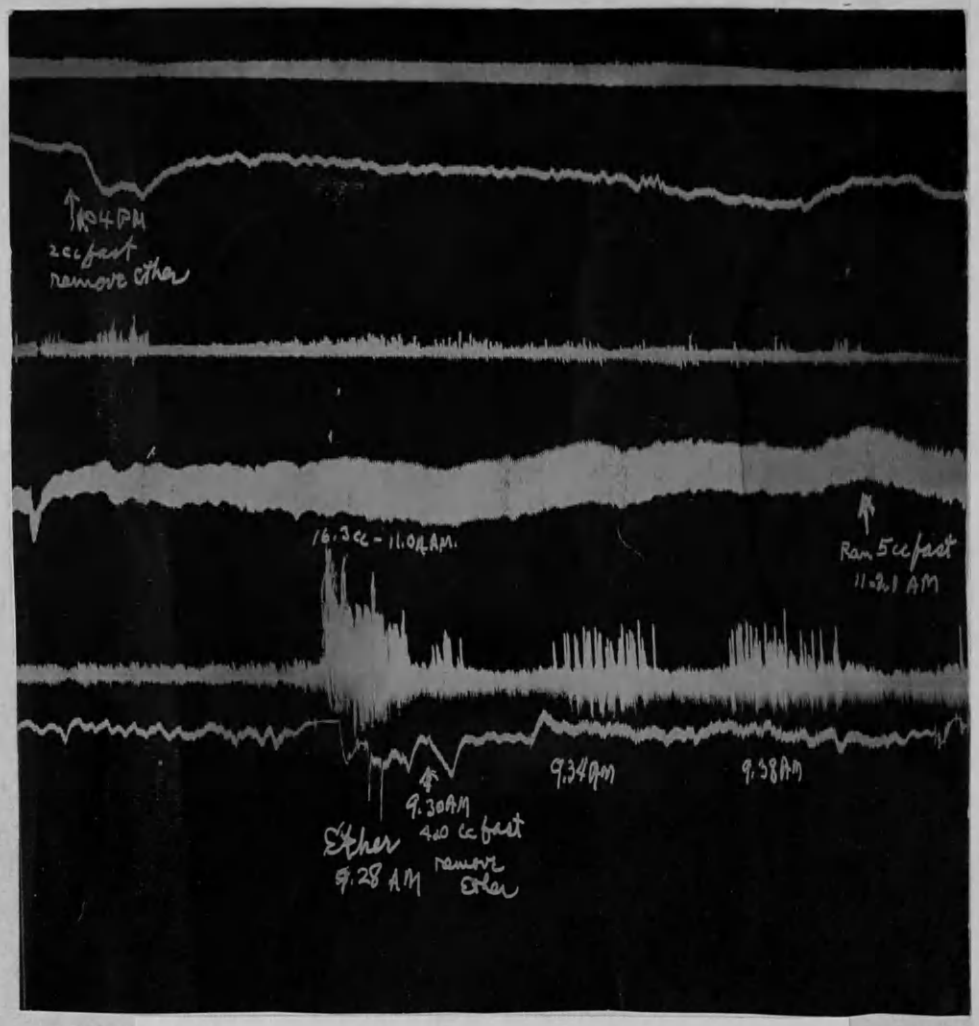
[8]It was necessary to use 10% alcohol since p-fluoroacetanilid was only slightly soluble in water.



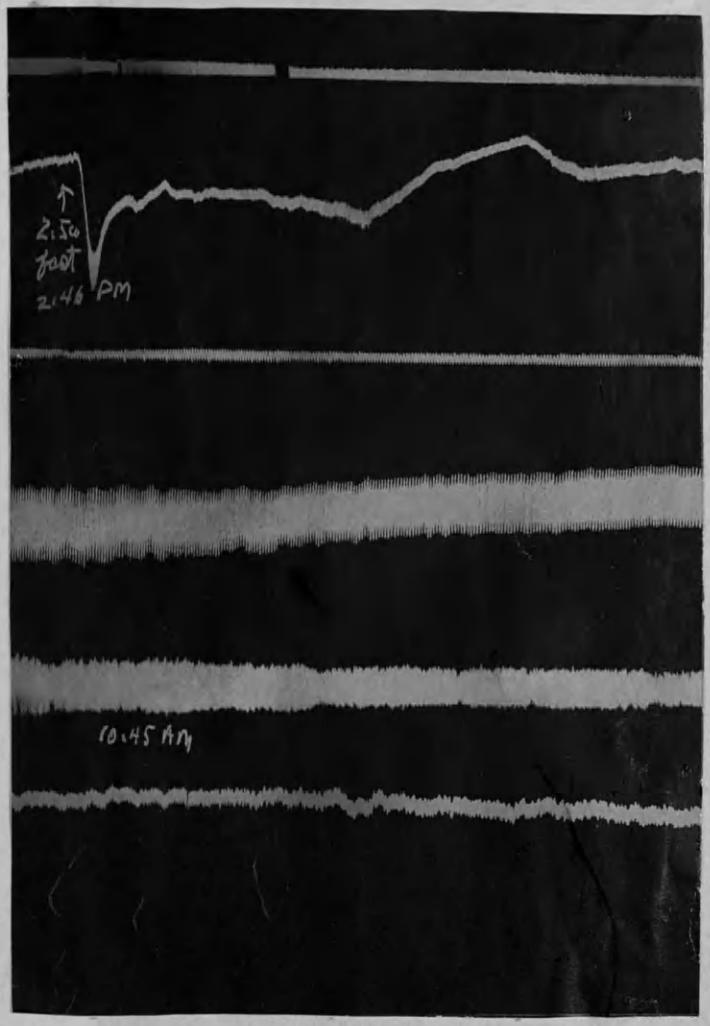


Fig. 1.—The upper tracing of each pair shows respiration, the lower shows carotid blood pressure (mercury manometer). The pairs reading from top to bottom are for *p*-fluoroacetanilid for 10 per cent alcohol alone and for acetanilid. Ether anesthesia. The first section was taken, 10 minutes after the beginning of the experiment and the second 65 minutes later.

No significant changes in the blood pressure as the result of the perfusion of p-fluoroacetanilid could be shown. A comparison of the tracings allows the following observations. After about 10 cc. of 10% alcohol had been perfused, the pulse pressure of the cat had doubled and, after about 40 cc., had tripled the original value. When the solution of either p-fluoroacetanilid or of acetanilid was run in, the pulse pressure remained unchanged or increased by about one-half its original value, remaining so until the animal was near death. This seemed to indicate that both p-fluoroacetanilid and acetanilid partly prevented the increase in pulse pressure that would have resulted from the administration of the alcohol alone. Another minor difference which may be pointed out was the behavior upon rapid injection of the solution. When 3 cc. of the solution of p-fluoroacetanilid or of acetanilid was injected rapidly, a sharp transient fall of blood pressure occurred. Under similar conditions, 5 cc. of 10% alcohol caused only a slight fall in blood pressure. Herz (125) stated that acetanilid, when injected intravenously in 35% alcohol, produced a tracing identical with that given by 35% alcohol alone. Apparently the effect of the 35% alcohol was sufficient to obscure any effect the acetanilid may have had.

The experiments on acute toxicity were carried out with cats of both sexes in the weight range of 2.0 to 2.5 kg. The doses were given orally by capsule. A number of observations showed that cats given p-fluoroacetanilid appeared to develop toxic symptoms (stiffening of the hind legs, loss of sense of balance) more quickly than those given acetanilid. Emesis and defecation were often observed within 1 hour after the administration of the larger doses of either compound. In the advanced stages, there was a relaxation of the abdominal muscles. Near

death the animals lay down breathing heavily and the heart slowed and became irregular. Convulsions were sometimes observed. In all cases, the respiration failed first. The doses affected the liver function, the liver (on autopsy) showing a peculiar mottling. The cats receiving p-fluoroacetanilid generally exhibited less dilatation of the pupils and less salivation. Both compounds produced cyanosis.

There was little difference in the oral dose of p-fluoroacetanilid and of acetanilid necessary to produce death. Acetanilid regularly produced death (5 out of 5 cats) when given orally in capsules containing 0.25 g. per kg. cat, while p-fluoroacetanilid was fatal in all cases in a dose of 0.275 g. per kg. Helms (126) stated that he used all the usual laboratory animals and that doses of 1000 mg. acetanilid failed to produce death in any case. Table II shows the results of the toxicity experiments.

TABLE II

SUMMARY OF THE EXPERIMENTS ON THE TOXICITY OF p-FLUOROACETANILID AND ACETANILID

Dose in g./kg. cat	p-Fluoroacetanilid	Acetanilid
0.033	R*	R R R R
0.090	R	R R
0.200	R D R D	R R D D
0.225	R D D R D R D D	D R D D R R D
0.250	R D R D R R D	D D D D D
0.275	D D D	
0.30	D D	

* R indicates recovery, D death.

Each letter indicates the result on one animal. No recovered animals were used again.

To determine whether there was any difference in chronic toxicity, some cats were given daily doses of p-fluoroacetanild or of acetanilid by capsule. The daily administration of 0.03 g. per kg. cat (one-eighth the dose of acetanilid found to be fatal) of p-fluoroacetanilid or acetanilid to separate cats for 3 weeks produced very little noticeable effect. There was practically no loss in weight by either of the cats. The dose was doubled for a period of 5 weeks. Neither cat showed much change, although the cat receiving acetanilid lost a little more weight than the cat receiving p-fluoroacetanilid. The daily dose was raised to 0.125 g. per kg. when the decline in weight became more evident and the cats were definitely depressed at all times. Five days later, the cat receiving p-fluoroacetanilid died. The kidneys showed extensive hemorrhage, the liver the characteristic mottled appearance. The teeth showed no spots. The cat receiving acetanilid lived for 34 days. Its liver and kidneys showed similar effects. A pair of cats was started with a daily dose of 0.125 g. per kg. of both compounds. The cat receiving p-fluoroacetanilid died after the second dose, the cat receiving acetanilid after the fifth dose. Another cat given the same daily dose of p-fluoroacetanilid died after the third dose.

The antipyretic activities of the compounds were compared in the following manner. Cats weighing from 1.5 to 3 kg. were given an intraperitoneal injection of 10 cc. of a 5% solution of dried egg albumin in normal saline solution at 9 A. M. The rectal temperature was taken before the injection and every hour until noon. At 12 noon, a capsule containing the desired dose of acetanilid or p-fluoroacetanilid (0.025 g. per kg. cat) was given orally. The rectal temperature was taken every half hour for 2 hours and then every hour for 2 hours. In all cases, the fall in temperature produced by acetanilid was quite marked

while that produced by p-fluoroacetanilid was insignificant. In order to rule out any resistance of the cats to one or the other of the compounds, the doses of the compounds were reversed after a suitable rest period. Since the doses of p-fluoroacetanilid uniformly produced little or no fall in rectal temperature, the dose in a series of trials was raised to 0.05 g. per kg. cat. This dose of p-fluoroacetanilid elicited light toxic symptoms in some of the cats but still produced no significant fall in temperature. Figure 2 shows the temperature curves obtained for one cat since they were more or less typical of the results obtained.

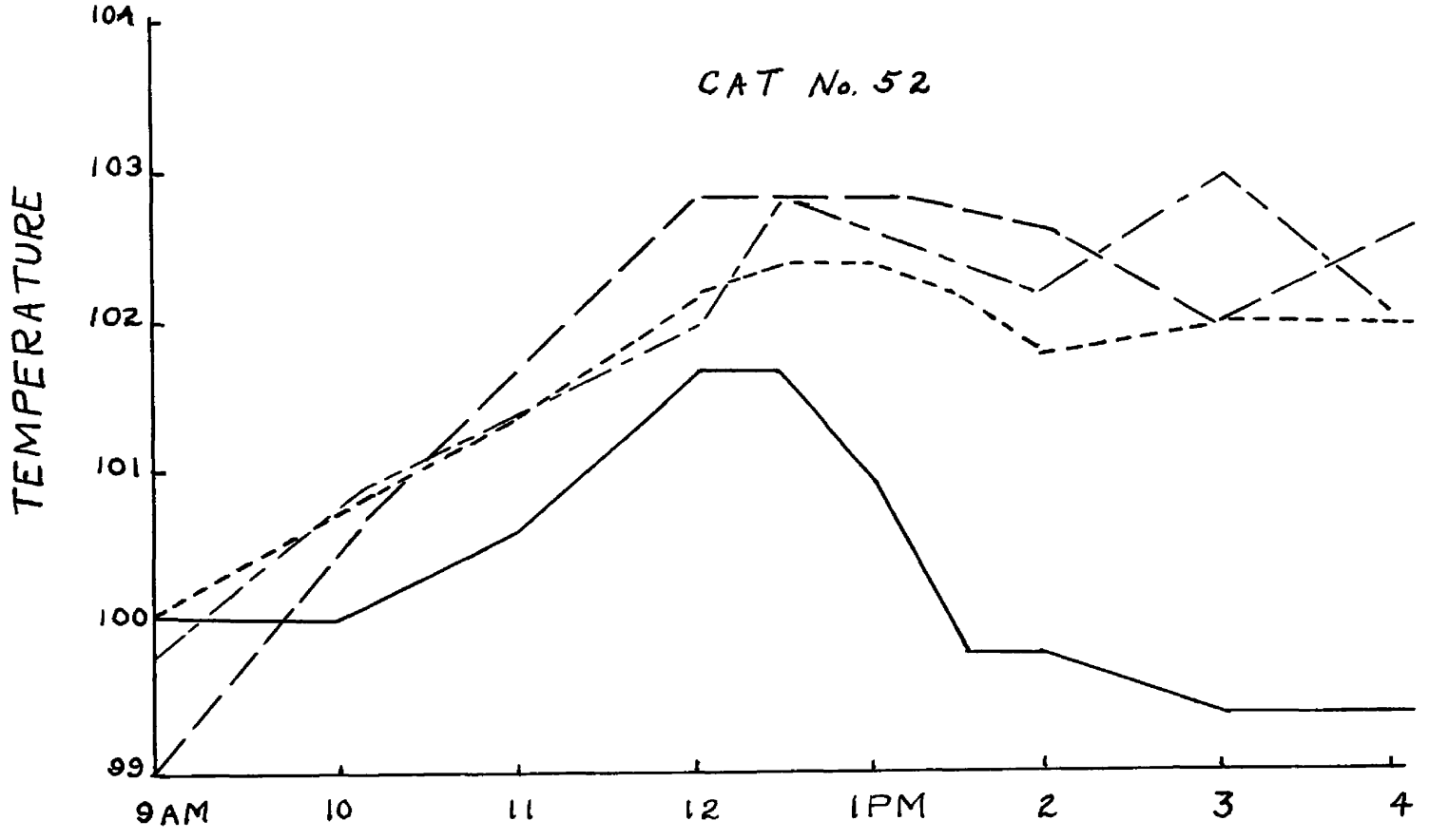


Figure 2

The course of the fever is shown by — — — —, the effect of a dose of 0.025 g. per kg. acetanilid by ————, the effect of the same dose of p-fluoroacetanilid by - - - - - - - -, and of 0.05 g. per kg. p-fluoroacetanilid by — - — - —.

In this connection, it should be noted that it was often possible

to use the same cat on successive days or sometimes on the second day after a previous use. However, if a week or more intervened between the trials on the cat, the fever produced was not constant and the results could not be relied upon. To avoid this difficulty when the above procedure became impractical, the following modification was adopted. Five cats were injected with 10 cc. of 5% solution of albumin and the temperatures recorded as before. At noon, capsules of m-fluoroacetanilid (0.025 g. per kg.) were given to all of the cats. The rectal temperatures were recorded at the intervals indicated before and the average temperatures of the five cats plotted. The same procedure was followed with p-chloroacetanilid. Figure 3 indicates the results obtained. For the purpose of comparison, the data obtained for p-fluoroacetanilid and for acetanilid have been recalculated on the basis just described and are shown also.

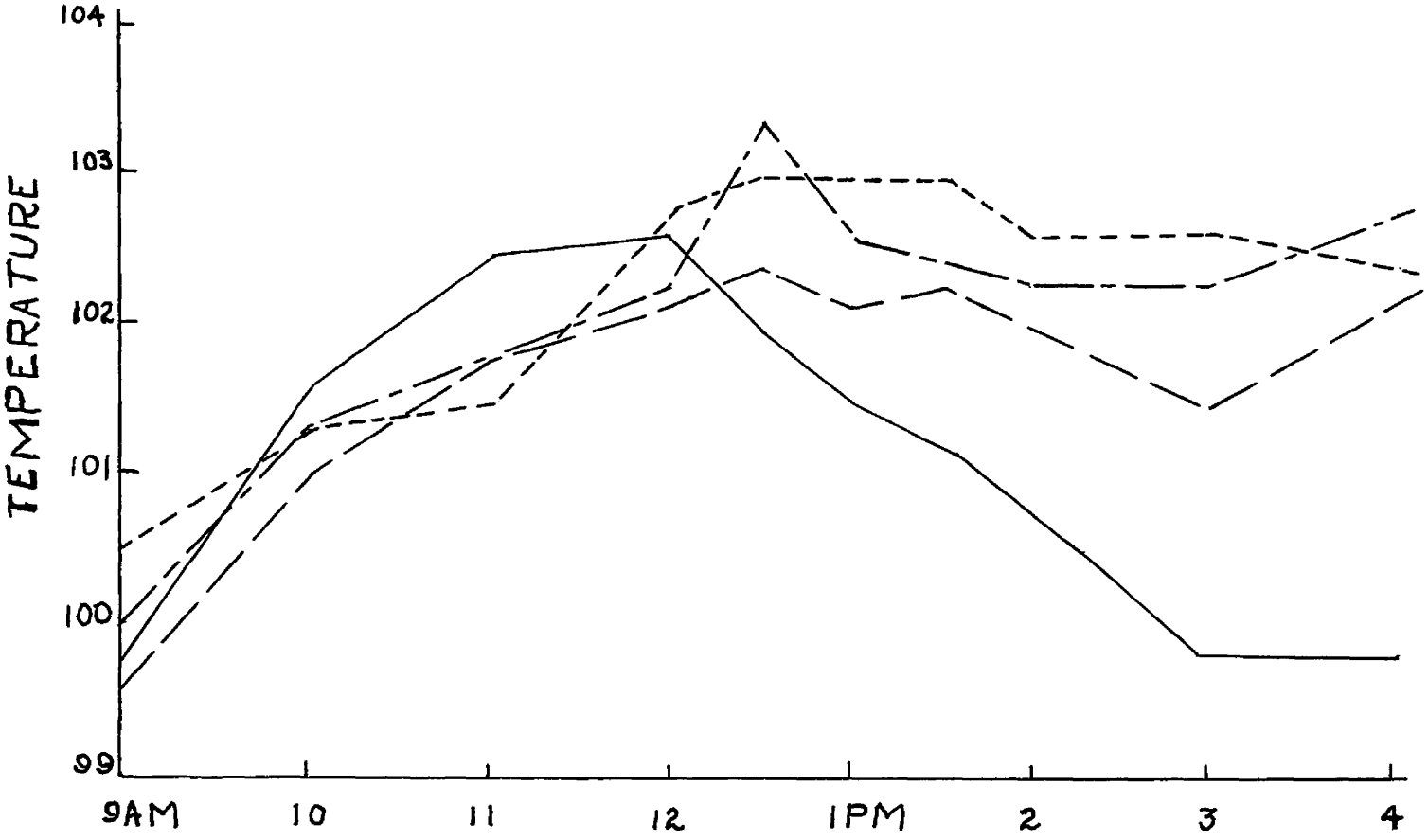


Figure 3

The effect of 0.025 g. per kg. acetanilid is shown by ————, the effect of the same dose of p-fluoroacetanilid by- - - - - - - -, of the same dose of m-fluoroacetanilid by—— - —— - ——, and of the same dose of p-chloroacetanilid by —— —— —— .

The slight difference in the acute toxicity of p-fluoroacetanilid as compared to acetanilid may be dependent on the lower degree of solubility of the former. Apparently a certain degree of tolerance is established after repeated dosage as is evident from the fact that cats having received small doses of both compounds for a period of several weeks, withstood the doses of 0.125 g. per kg. for a longer time than cats started out with this dose. Substitution of fluorine or chlorine in the benzene nucleus of acetanilid very markedly reduced the antipyretic activity of acetanilid and in the case of p-fluoroacetanilid, scarcely affected the toxicity.

BACTERIOLOGY

Literature Survey

In addition to the use of inorganic fluorine compounds as antiseptics, for example sodium fluoborate (127), several organic fluorine compounds have been recommended from time to time.

Moissan (128) claimed antiseptic properties for ethyl fluoride, Chabrié (129) showed that methyl fluoride would sterilize urine from pyogenic infections.

A compound for which much has been claimed is p,p'-difluorodiphenyl (130). The compound is non-toxic to rabbits in doses of 2 g. per day over a period of 5 days. Bacteriological studies in vitro showed that difluorodiphenyl in concentrations varying from 1 to 100 to 1 to 20 did not kill Streptococcus pyogenes, Staphylococcus aureus, cholera vibrios, diphtheria bacilli or anthrax bacilli. However, when applied to wound surfaces, burns, suppurating ulcers and other epidermal infections, decidedly beneficial results and a rapid regeneration of the epidermis were claimed. Valentiner (131) commented upon the retarding effect of p,p-difluorodiphenyl, p-fluorophenetole and fluoropseudocumol on the growth of bacteria. These compounds have also been shown to diffuse through animal membranes easily. "Antitussin" (p,p'-difluorodiphenyl in lanolin) was recommended for whooping cough and throat and chest congestions in general (84, 85, 131, 132). "Epidermin" is an ointment containing difluorodiphenyl and fluoroxylol in lanolin (84, 131) and is recommended for wounds and burns. A mixture of p,p'-difluorodiphenyl and p-fluorophenetole in lanolin (84, 131) is recommended for ischias and rheumatism in its

various forms.

Fluoropseudocumol, fluoroform, 1-fluoro-naphthalene-5-sulfonic acid and fluoroacetic acid have been used as protectives against moths in skins and wool (7). The compounds resulting when acetylene, allylene and oleic acid are treated with gaseous HF are recommended as insecticides and disinfectants (133). Mercury derivatives of benzotrifluoride which also contain a nitro, hydroxy or carboxyl group on the ring are claimed to have antiseptic and therapeutic value (6). Organic fluorine compounds which also contain a carboxyl group are useful as fungicides, insecticides and disinfectants (134). Various fluorinated aliphatic hydrocarbons as fluorinated mineral oils, fluorinated amylene, etc. are described as fungicides (135).

Suter, Lawson and Smith (4) prepared and tested the germicidal properties of a series of 4-fluoro-2-alkylphenols, the alkyl radicals being, ethyl, n-propyl, n-butyl, n-amyl and n-hexyl. The fluoroalkylphenols were found to be more efficient germicides under the conditions investigated than the alkyl phenols but were not so active as the corresponding chloroalkylphenols.

M. C. Hart and H. P. Anderson (66) have prepared and tested some 21 mercury compounds for their antiseptic properties. Among these was 2(?)-acetoxymercuri-p-chlorphenol prepared from the action of mercuric acetate on p-chlorophenol. The compound was inhibitory to S. aureus in 5 min. at 1-10,000. However, no direct comparison can be made with the bacteriostatic activity of 2(?)-acetoxymercuri-p-fluorophenol since the methods of testing differed.

Experimental

Some bacteriological tests on the mercurials have been carried out. The solubilities in water of most of the mercurials prepared were very low. However, 4-fluoro-2-acetoxymercuriphenol was soluble to the extent of 1 to 1000 in water at room temperature and so could be used in solution. Suspensions of the other compounds were prepared in the following manner: a weighed quantity of mercurial was dissolved in the smallest possible amount of boiling alcohol or acetone and then poured slowly into sufficient of a well-stirred 0.5% suspension of tragacanth in distilled water to make a 1 to 1000 suspension. The tragacanth retarded the precipitation of the mercurial in large flakes and the suspension so produced did not settle readily. The suspension was stirred for about a half hour in a warm water bath to drive off most of the alcohol or acetone and then made up to the required volume with water.

The compounds have been tested against the Food and Drug Administration strains of Staphylococcus aureus (#289) and Eberthella typhi (Hopkins strain) in water and undiluted serum. p-Chlorophenylmercuric chloride was selected as a standard against which to judge the effect of the presence of fluorine on the antiseptic properties of the mercurials. In order to maintain conditions as uniform as possible, all six compounds were tested on the same day using the same culture of organisms within a period of about 3 hours.

The tests in water were carried out as follows: shortly before use, 100 cc. of the dilutions, shown by preliminary tests to fall in the proper range, were prepared from the stock 1 to 1000 suspensions. One

cc. of a 24 hour broth culture was inoculated into 100 cc. of the dilution, the suspension shaken vigorously and 1 cc. samples plated in nutrient agar after 1, 10, and 60 minute exposures. The plates were observed after 48 hours incubation at 37° C. and all negative plates reincubated for 48 hours to be certain that no growth would develop. The negative plates were streaked with Staphylococcus aureus or Eberthella typhi and the presence of growth indicated a lack of bacteriostatic action. At the same time the test was being run, the organisms in the inoculum were likewise counted on nutrient agar plates.

Tables III and IV show the results of the tests using aqueous suspensions of the mercurial against Staphylococcus aureus and Eberthella typhi respectively. The figures in the tables indicate the number of colonies, the letter I indicating profuse growth which could not be counted. As may be seen from the tables, the number of colonies found at a given concentration decreased with the increased time of exposure, demonstrating bactericidal action. The zeros that appear indicate no growth, probably due to bacteriostatic action as a result of mercurial carried over into the plate. It will be shown later that the concentrations used did not kill all of the organisms.

TABLE III

BACTERIOSTATIC AND BACTERICIDAL PROPERTIES OF FLUORINATED AROMATIC MERCURIALS

Compound	Exposure	Dilution of Mercurials in Water				
		$1x10^5$	$3x10^5$	$5x10^5$	$7x10^5$	$1x10^6$
4-fluorophenyl-mercuric chloride	1 min.	4*	4000	I**	I	I
	10 min.	0	3500	I	I	I
	60 min.	0	200	200	I	I
3-fluorophenyl-mercuric chloride	1 min.	0	I	5000	I	I
	10 min.	0	110	I	I	I
	60 min.	0	0	1	I	I
2-fluorophenyl-mercuric chloride	1 min.	0	0	0	I	I
	10 min.	0	0	300	450	I
	60 min.	0	0	0	0	30
4-chlorophenyl-mercuric chloride	1 min.	0	2	0	1800	I
	10 min.	0	0	105	300	I
	60 min.	0	0	1	0	1
4-fluoro-2-acetoxymercuri-phenol	1 min.	0	0	1000		
	10 min.	0	2	45		
	60 min.	0	0	0		
		$1x10^4$	$3x10^4$	$5x10^4$	$7x10^4$	$1x10^5$	$3x10^5$
4-fluoro-3-chloromercuri-benzoic acid	1 min.	0	0	2	65	I	I
	10 min.	0	0	0	3	800	I
	60 min.	0	0	3	0	0	1500

* - Number of colonies ** - Innumerable colonies

500,000,000 S. aureus (F. D. A. strain) inoculated into 100 cc. of dilutions of mercurial. One cc. removed and plated in nutrient agar at times indicated. All tests conducted at room temperature.

TABLE IV

BACTERIOSTATIC AND BACTERICIDAL PROPERTIES OF FLUORINATED AROMATIC MERCURIALS

Compound	Exposure	Dilutions of Mercurials in Water					
		$1x10^4$	$1x10^5$	$3x10^5$	$5x10^5$	$7x10^5$	$1x10^6$
4-fluorophenyl-mercuric chloride	1 min.	0	0	I*	I	I	I
	10 min.	0	2**	135	I	I	I
	60 min.	0	1	0	95	175	8
3-fluorophenyl-mercuric chloride	1 min.	0	I	I	I	I	I
	10 min.	0	0	260	2300	3500	I
	60 min.	0	0	0	165	1600	0
2-fluorophenyl-mercuric chloride	1 min.	0	I	15,000	I	I	I
	10 min.	0	500	260	4000	I	5000
	60 min.	0	0	0	110	575	240
4-chlorophenyl mercuric chloride	1 min.	0	1	5	5000	I	I
	10 min.	0	0	5	12	192	3000
	60 min.	0	0	0	0	1	9
4-fluoro-2-acetoxymercuri-phenol	1 min.	0	1000	I	I	I	I
	10 min.	0	9	85	50	3500	I
	60 min.	0	3	7	9	4000	I
		$1x10^4$	$3x10^4$	$5x10^4$	$7x10^4$	$1x10^5$	$3x10^5$
4-fluoro-3-chloromercuri-benzoic acid	1 min.	0	0	750	I	I	I
	10 min.	0	0	41	3	27	3100
	60 min.	0	0	0	0	0	175

* - Innumerable colonies ** - Number of colonies

1,200,000,000 E. typhi (F. D. A. strain) inoculated into 100 cc. of dilutions of mercurial. One cc. removed and plated in nutrient agar at times indicated. All tests conducted at room temperature.

Using the same method as described for the preparation of the aqueous dilutions, stock suspensions of the compounds except 4-fluoro-2-acetoxy-mercuriphenol were prepared in undiluted beef serum sterilized by Seitz filtration. The phenol derivative was soluble in serum to the extent of 1 to 1000 without causing precipitation and so could be used in solution.

The serum dilutions were then incubated for 24 hours at 37° to be certain that they were sterile. The tests were set up in a manner quite similar to those run in water, maintaining as nearly as possible, the same proportions of organisms. Five cc. of the serum dilutions prepared from the stock suspensions were inoculated with 0.1 cc. of a 24 hour broth culture, and 1 cc. samples plated after 1, 10 and 60 min. exposures. The effective dilutions of the compounds in serum were lower than in water. Tables V and VI show the results of the tests in serum against Staphylococcus aureus and Eberthella typhi respectively. The explanation of the characters is the same as for the previous tables.

TABLE V

BACTERIOSTATIC AND BACTERICIDAL PROPERTIES OF FLUORINATED AROMATIC MERCURIALS

Compound	Exposure	Dilution of Mercurials in Serum					
		$1x10^3$	$1x10^4$	$3x10^4$	$5x10^4$	$7x10^4$	$1x10^5$
4-fluorophenyl-mercuric chloride	1 min.	0	0	600*	2000	3500	I**
	10 min.	0	0	210	1300	3000	I
	60 min.	0	0	38	650	3000	I
3-fluorophenyl-mercuric chloride	1 min.	0	0	0	120	2000	I
	10 min.	0	0	3	95	1300	I
	60 min.	0	0	0	10	700	I
2-fluorophenyl-mercuric chloride	1 min.	0	500	300	2000	I	I
	10 min.	0	225	100	2500	I	I
	60 min.	0	230	800	2000	I	I
4-chlorophenyl-mercuric chloride	1 min.	0	0	I	I	I	I
	10 min.	0	7	800	I	I	I
	60 min.	0	2	600	I	I	I
4-fluoro-2-acetoxymercuri-phenol	1 min.	0	240	2500	I		
	10 min.	0	130	370	I		
	60 min.	0	35	I	I		
		$1x10^3$	$1x10^4$	$3x10^4$			
4-fluoro-3-chloromercuri-benzoic acid	1 min.	0	I	I			
	10 min.	0	I	I			
	60 min.	0	I	I			

* - Number of colonies ** - Innumerable colonies

25,000,000 S. aureus (F.D.A. strain) inoculated into 5 cc. dilutions of mercurial in beef serum. One cc. removed and plated in nutrient agar at times indicated. All tests conducted at room temperature.

TABLE VI

BACTERIOSTATIC AND BACTERICIDAL PROPERTIES OF FLUORINATED AROMATIC MERCURIALS

Compound	Exposure	Dilutions of Mercurials in Serum $1x10^3$	$3x10^3$	$5x10^3$	$7x10^3$
4-fluorophenyl-mercuric chloride	1 min.	0	0	I*	I
	10 min.	0	0	1500**	I
	60 min.	0	0	500	I
3-fluorophenyl-mercuric chloride	1 min.	0	700	I	I
	10 min.	0	0	I	I
	60 min.	0	0	1450	I
2-fluorophenyl-mercuric chloride	1 min.	0	0	I	I
	10 min.	0	200	I	I
	60 min.	0	0	I	I
4-chlorophenyl-mercuric chloride	1 min.	0	0	I	I
	10 min.	0	8	I	I
	60 min.	0	10	I	I
4-fluoro-2-acetoxymercuri-phenol	1 min.	0	0	I	I
	10 min.	0	0	I	I
	60 min.	0	0	450	I

Compound	Exposure	$5x10^2$	$7x10^2$	$1x10^3$	$3x10^3$	$5x10^3$	$7x10^3$
4-fluoro-3-chloromercuri-benzoic acid	1 min.	0	0	0	0	I	I
	10 min.	0	0	0	0	I	I
	60 min.	0	0	0	0	I	I

* Innumerable colonies ** - Number of colonies

90,000,000 E. typhi (F. D. A. strain) inoculated into 5 cc. dilutions of mercurials in beef serum. One cc. removed and plated in nutrient agar at times indicated. All tests conducted at room temperature.

In order to summarize the results so far, Table No. VII was compiled, showing the results of the tests against both organisms in water and serum. The compounds are listed in order of decreasing activity down the columns. The Roman numeral I is assigned to the most effective

compound, II to the next most effective, etc. In water, 4-chlorophenylmercuric chloride was the most effective against either S. aureus or E. typhi. In serum, the positions were reversed, the 4-chlorophenylmercuric chloride being less effective than the three fluorophenylmercuric chlorides.

TABLE VII

RELATIVE BACTERICIDAL AND BACTERIOSTATIC PROPERTIES OF FLUORINATED AROMATIC MERCURIALS

Dilutions in Water

	Staphylococcus aureus		Eberthella typhi
I	4-Chlorophenylmercuric chloride	I	4-chlorophenylmercuric chloride
II	2-fluorophenylmercuric chloride	II	4-fluorophenylmercuric chloride
III	4-fluoro-2-acetoxymercuriphenol	III	3-fluorophenylmercuric chloride
IV	4-fluorophenylmercuric chloride	IV	4-fluoro-2-acetoxymercuriphenol
V	3-fluorophenylmercuric chloride	V	2-fluorophenylmercuric chloride
VI	4-fluoro-3-chloromercuribenzoic acid	VI	4-fluoro-3-chloromercuribenzoic acid

Dilutions in Serum

I	3-fluorophenylmercuric chloride	I	4-fluorophenylmercuric chloride
II	4-fluorophenylmercuric chloride	II	4-fluoro-2-acetoxymercuriphenol
III	2-fluorophenylmercuric chloride	III	3-fluorophenylmercuric chloride
IV	4-chlorophenylmercuric chloride	IV	2-fluorophenylmercuric chloride
V	4-fluoro-2-acetoxymercuriphenol	V	4-chlorophenylmercuric chloride
VI	4-fluoro-3-chloromercuribenzoic acid	VI	4-fluoro-3-chloromercuribenzoic acid

If the numbers in Table VII indicating the positions of the com-

pounds in all of the tests are averaged, the compounds can then be arranged in the following order of decreasing activity.

TABLE VIII

COMPOSITE VALUE FOR BACTERIOSTATIC AND BACTERICIDAL PROPERTIES AGAINST *E. TYPHI* AND S. *AUREUS* IN WATER AND SERUM

Compound	Value
4-fluorophenylmercuric chloride	2.25
4-chlorophenylmercuric chloride	2.75
3-fluorophenylmercuric chloride	3.00
2-fluorophenylmercuric chloride	3.50
4-fluoro-2-acetoxymercuriphenol	3.50
4-fluoro-3-chloromercuribenzoic acid	6.00

It should be emphasized that the above is an entirely arbitrary method of comparison combining the results of a variation of the four factors (water, serum, and the two organisms). Taking all conditions into consideration, the 4-fluorophenylmercuric chloride is the most effective of those compounds studied.

An attempt was made to establish the dilutions of the compounds which were bactericidal at room temperature. Fifty cc. of the aqueous suspensions were inoculated with 0.5 cc. of a 24 hour broth culture of the organism and after 1 hour, two (4 mm.) loopfuls of the suspensions were transferred to 100 cc. of sterile broth. The bottles of broth were incubated at 37° C. for one week. The bottles were observed daily during a week of incubation. The period of one week was chosen as a practical time limit for the experiment. Two bottles were inoculated from each dilution and are indicated separately. Table IX shows the

results obtained when E. typhi was used.

TABLE IX

BACTERICIDAL PROPERTIES OF FLUORINATED AROMATIC MERCURIALS

Compound	Dilutions of mercurials in water				
	$1x10^3$	$3x10^3$	$5x10^3$	$7x10^3$	$9x10^3$
4-fluorophenyl mercuric chloride	- -	- -	- -	- -	- -
3-fluorophenyl-mercuric chloride	- -	- +*	- -	- -	- +*
2-fluorophenyl-mercuric chloride	- -	- -	- +*	- +	- -
4-chlorophenyl-mercuric chloride	- -	- -	- +*	- -	- -
2-acetoxymercuri-4-fluorophenol	- +*	+ +	- -	- +*	- +*
3-chloromercuri-4-fluorobenzoic acid	+ +	+* +*	- -	+* +*	+ -

-Indicates lack of growth; indicates growth; * staphylococci found but no E. typhi.

330,000,000 E. typhi (F. D. A. strain) inoculated into 50 cc. of dilutions of mercurials. Two loopfuls (4 mm.) inoculated into 100 cc. of broth after one hour exposure. All tests conducted at room temperature.

At the end of the week, the negative bottles were inoculated with E. typhi and within 24 hours were cloudy, proving that the mercurial transferred in the inoculation exhibited no bacteriostatic effect. These results indicate that the negative plates shown in tables III to VI were in most cases probably due to the bacteriostatic conditions produced by small amounts of mercurial carried over into the plates.

When S. aureus was used as the test organism, the results were not

very consistent. At the end of the week period of incubation, almost every bottle showed growth. The possibility of air-borne contamination by staphylococci made it impossible to determine which bottles had been accidentally contaminated during the transfers, and which had become cloudy as the result of the inoculated organisms.

The testing technique described in this paper was then applied to two mercurials obtainable on the market. The same two organisms were used and 4-fluorophenylmercuric chloride was run at the same time under the same conditions. Table X indicates the results obtained. It will be seen that when the described technique was used, 4-fluorophenylmercuric chloride compares favorably with merthiolate and metaphen against S. aureus and E. typhi in water dilutions.

TABLE X

COMPARISON OF THE BACTERIOSTATIC AND BACTERICIDAL PROPERTIES OF METAPHEN, MERTHIOLATE AND 4-FLUOROPHENYLMERCURIC CHLORIDE

Eberthella typhi $1x10^9$ organisms inoculated

Compound	Exposure	Dilutions of the Mercurials in Water						
		$7x10^4$	$1x10^5$	$3x10^5$	$5x10^5$	$7x10^5$	$1x10^6$	$3x10^6$
4-fluorophenyl-mercuric chloride	1 min.		0*	I**	I	I	I	
	10 min.		0	I	I	I	I	
	60 min.		0	700	500	77	I	
Merthiolate	1 min.	0	0	I	27	103	I	I
	10 min.	0	0	18	40	I	I	I
	60 min.	0	0	50	121	I	I	I
Metaphen	1 min.		600	500	I	I	I	I
	10 min.		35	17	I	400	I	I
	60 min.		0	0	60	14	350	I

Staphylococcus aureus $6.5x10^8$ organisms inoculated

Compound	Exposure	Dilutions of the Mercurials in Water						
		$1x10^5$	$3x10^5$	$5x10^5$	$7x10^5$	$1x10^6$	$2x10^6$	$3x10^6$
4-fluorophenyl mercuric chloride	1 min.	0	0	105	500	0		5000
	10 min.	0	0	0	1	7		I
	60 min.	0	0	0	0	6		1
Merthiolate	1 min.		0	I	2	I	I	I
	10 min.		0	I	0	I	I	I
	60 min.		0	5	I	12	6	I
Metaphen	1 min.	0	0	I	8	I		I
	10 min.	0	0	0	I	900		I
	60 min.	0	0	0	7	0		I

* - Number of colonies ** - Innumerable colonies

One cc. of the cultures indicated inoculated into 100 cc. of dilutions of mercurial. One cc. removed and plated in nutrient agar at times indicated. All tests conducted at room temperature.

The 4-fluorophenylmercuric chloride was compared to merthiolate and metaphen in serum, using the technique described under the serum tests.

The suspension of 4-fluorophenylmercuric chloride was prepared as before, the merthiolate required to prepare a 1 to 1000 solution was dissolved in undiluted serum, and the metaphen solution was prepared by mixing equal amounts of 1 to 500 solution (aqueous) and serum. Table XI shows the results of these tests.

TABLE XI

COMPARISON OF THE BACTERIOSTATIC AND BACTERICIDAL PROPERTIES OF METAPHEN, MERTHIOLATE AND 4-FLUOROPHENYLMERCURIC CHLORIDE

Eberthella typhi - $1x10^8$ organisms inoculated

Compound	Exposure	Dilutions of the Mercurials in Serum $1x10^3$	$1.5x10^3$	$3x10^3$	$5x10^3$	$7x10^3$	$1x10^4$	$3x10^4$
4-fluorophenyl-mercuric chloride	1 min.	0	a	0	58[b]	8000	I[c]	
	10 min.	0		0	16	5000	I	
	60 min.	0		0	2	3000	I	
Merthiolate	1 min.		0	0	0	0	0	I
	10 min.		0	0	0	0	0	I
	60 min.		0	0	0	0	0	I
Metaphen	1 min.		0	0	100	I	I	
	10 min.		0	0	I	I	I	
	60 min.		0	0	0	I	I	

Staphylococcus aureus - $6x10^7$ organisms inoculated

Compound	Exposure	Dilutions of the Mercurials in Serum $5x10^3$	$1x10^4$	$3x10^4$	$5x10^4$	$7x10^4$	$1x10^5$
4-fluorophenyl-mercuric chloride	1 min.	25	0	500	2650	6500	I
	10 min.	0	0	4750	4000	6000	I
	60 min.	0	5	2250	1300	4000	I
Merthiolate	1 min.	0	3	2100	I	I	
	10 min.	5	6	1100	I	I	
	60 min.	5	0	1000	I	I	
Metaphen	1 min.	60[d]	I	I	I	I	
	10 min.	I	I	I	I	I	
	60 min.	225[d]	I	I	I	I	

a - Dilution not run b - Number of colonies c - Innumerable colonies d - Large colonies only; profuse pin point colonies.

One-tenth cc. of cultures indicated inoculated into 5 cc. of dilutions of mercurial. One cc. removed and plated in nutrient agar at times indicated. All tests conducted at room temperature.

A method of testing mercurials which gives indications of both bactericidal and bacteriostatic actions has been employed. A solution or suspension of the mercurial to be tested was inoculated with the organisms and 1 cc. of the mixture plated in nutrient agar after varying times of exposure. The number of colonies growing out varied directly with the dilution and, at a given dilution, varied inversely with the time of exposure. The fact that the number of colonies decreased with an increasing time of exposure, indicated that the bactericidal action before plating had probably reduced the number of viable organisms. A consideration of the broth bottle tests for complete bactericidal action indicated that those plates showing no growth at all apparently contained a sufficient amount of mercurial to be bacteriostatic for the viable organisms remaining.

When the three fluorophenylmercuric chlorides are compared to p-chlorophenylmercuric chloride by the methods described, two interesting facts may be pointed out. When the tests were run in water, the 4-chloro- compound was slightly more effective than the fluoro compounds; in serum it was less effective. When the tests against both organisms in water and serum are considered together, the three fluoro compounds lined up in the order of decreasing activity as follows: para, meta, ortho.

p-Fluoro-o-acetoxymercuriphenol was just slightly less active than the fluorophenylmercuric chlorides. p-Fluoro-m-chloromercuribenzoic acid was the least active compound under any of the conditions investigated. The effective concentrations of it were generally five times as great as for 4-fluorophenylmercuric chloride.

Since 4-fluorophenylmercuric chloride appeared to be the most

effective compound when all factors were considered, it was selected for comparison against some known mercurials by the method used in this paper. In both water and serum 4-fluorophenylmercuric chloride proved to be slightly more effective against S. aureus than merthiolate or metaphen. It was less effective against E. typhi than merthiolate but equally as effective as metaphen.

Summary

1. Mercurials prepared from the replacement of the diazonium group by mercury are generally more easily purified and the melting points higher than when the same compounds are prepared by other methods.

2. The melting points of the three isomeric fluorophenylmercuric chlorides are higher than for the corresponding chlorophenylmercuric chlorides.

3. Evidence is presented to show that p-fluorobenzoic acid, when mercurated directly using mercuric acetate in glacial acetic acid, yields 3-acetoxymercuri-4-fluorobenzoic acid.

4. The direct mercuration of fluorobenzene with mercuric acetate in alcohol or glacial acid was not entirely satisfactory. However, about a 10% yield of o-fluorophenylmercuric acetate was obtained when the reaction was run in glacial acetic acid.

5. The position taken by the metal on the mercuration of p-fluorophenol with mercuric acetate is not established with certainty, but the position ortho to the hydroxyl is suggested.

6. The introduction of fluorine in positions meta or para to the functional group of acetanilid destroys the antipyretic activity.

7. p-Fluoroacetanilid exhibits no greater acute toxicity for cats than does acetanilid.

8. The three fluorophenylmercuric chlorides and 2-acetoxymercuri-4-fluorophenol are highly bacteriostatic while 3-chloromercuri-4-fluorobenzoic acid is only about one-fifth to one-tenth as effective.

9. The mercurated benzoic acid in a dilution of 1 to 1000 and the four other fluorinated mercurials prepared, in dilutions of 1 to 9000 are completely bactericidal in one hour against E. typhi in water.

10. Dilutions of 4-chlorophenylmercuric chloride in water are slightly more effective against S. aureus and E. typhi than the fluoro-mercurials described.

11. Dilutions of the aromatic fluoro-mercurials (except the mercurated fluorobenzoic acid) are more effective in serum against the organisms tested than 4-chlorophenylmercuric chloride.

12. In both water and serum, 4-fluorophenylmercuric chloride is slightly more effective against S. aureus than either merthiolate or metaphen, but slightly less effective against E. typhi than merthiolate.

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