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## Synthesis and Toxicity of $N^1$-*p*-Fluorophenylsulfanilamide

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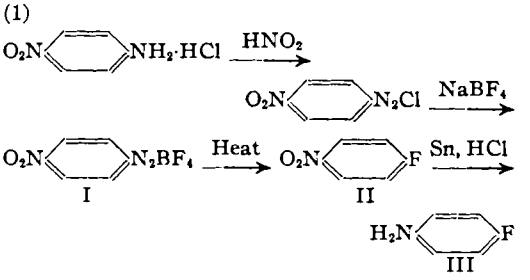
In reviewing the investigations of sulfanilamide derivatives, it was observed that the $N^1$-*p*-aminophenyl derivative has been reported to be twice as active and of the same order of toxicity as sulfanilamide (1). It was further noted at that time that there had been no reports of a fluorine derivative of sulfanilamide. In view of these facts the $N^1$-*p*-fluorophenylsulfanilamide was prepared as was the $N^1$-phenylsulfanilamide for purpose of comparison. As the investigation of these compounds was nearing completion, Suter and Weston (2) published a report on a series of fluorinated sulfanilamide derivatives among which was included the compound described in this paper.

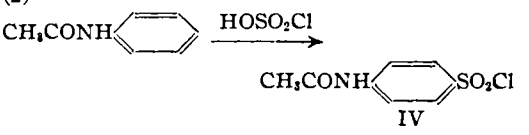
The work here reported gives a procedure for the preparation of $N^1$-*p*-fluorophenylsulfanilamide in better yield and higher state of purity (as judged by melting points) than that obtained by the earlier procedure of Suter and Weston (2). There are also included the results of the experiments thus far completed to determine the relative toxicities of sulfanilamide, $N^1$-phenylsulfanilamide and $N^1$-*p*-fluorophenylsulfanilamide.

The $N^1$-*p*-fluorophenylsulfanilamide was synthesized according to the following scheme:

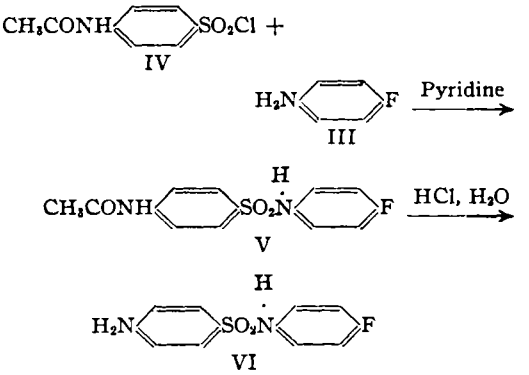
(1)




(2)




(3)




### EXPERIMENTAL

*I. p-Nitrobenzenediazonium Borofluoride.*—The compound was prepared by Dunker, Starkey and

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Jenkins (3) by an improved method taken from the work of Balz and Schiemann (4), involving the diazotization of *p*-nitroaniline in the presence of fluoboric acid. While better yields were obtained if the diazonium borofluoride was prepared directly using fluoboric acid, the method presented here obviates the necessity of preparing the fluoboric acid and utilizes the cheaper sodium fluoborate.

To 200 cc. of hydrochloric acid (sp. gr. 1.19) contained in a 2-l. beaker in an ice bath and stirred with a mechanical stirrer, were added 139 Gm. (1 mol.) of *p*-nitroaniline. The mixture was cooled to 5–10° C. While stirring vigorously, a solution of 69 Gm. (1 mol) of sodium nitrite in 150 cc. of water was added slowly from a separatory funnel, keeping the temperature of the mixture at 5–10° C. When the addition was complete, a cold filtered solution of 272 Gm. (2 mols) of sodium fluoborate in sufficient water was added to the solution of the diazonium chloride. The stirring was continued for fifteen minutes longer. The solid was filtered on a fritted glass funnel, washed once with alcohol and three times with ether and dried partially by drawing air through it. The resulting light yellow solid was dried over night in a desiccator over $P_2O_5$. The average yield was 92% of the theoretical based on the weight of *p*-nitroaniline taken.

*II. p-Fluoronitrobenzene.*—The preparation of aromatic fluorine compounds by the thermal decomposition of the diazonium borofluorides is described by Dunker and Starkey (5) who based their method on that developed by Schiemann and Pillarsky (6). The procedure used in this investigation was essentially the same as that employed by Dunker and Starkey. The chief difference consisted in carrying out this process at atmospheric rather than reduced pressure.

*III. p-Fluoroaniline.*—The *p*-fluoronitrobenzene was reduced in the usual manner by means of tin and hydrochloric acid.

*IV. p-Acetaminobenzenesulfonyl Chloride.*—Acetanilide was treated with chlorosulfonic acid and the acid chloride isolated by pouring the mixture on ice as described by Stewart (7). Although it was found best to use the crude damp material immediately because of decomposition, several attempts were made to prepare the stable pure product with indifferent success.

*V. $N^1$-p-Fluorophenyl-$N^4$-Acetylsulfanilamide.*—The procedure employed for the condensation of *p*-fluoroaniline with *p*-acetaminobenzenesulfonyl chloride was an adaptation of a more or less general method (1, 8, 9, 10). The amine is generally mixed with the acid chloride in molar proportions in some solvent in which both are soluble. The reaction is allowed to proceed at room temperature or slightly above. Care must be excerised in choosing the proper condensing agent for the hydrochloric acid liberated. When the amine is available and strongly basic, a mol in excess is used. When the amine is weakly basic, sodium bicarbonate or sodium acetate is used. When working with amino acids or nitro compounds, sodium hydroxide, potassium hydroxide or pyridine is necessary. Suter (2) prepared $N^1$-*p*-fluorophenyl-$N^4$-acetylsulfanilamide using water as a solvent and sodium carbonate as the condensing agent. Better yields were obtained when using acetone as solvent and pyridine as condensing agent as was done in the following procedure.

To a mixture of 5.6 Gm. (0.05 mol) of *p*-fluoroaniline, 12 Gm. (0.05 mol) of *p*-acetaminobenzenesulfonyl chloride and 40 cc. of acetone in a 1-l. round-bottomed flask with a reflux condenser, were added 8 cc. of pyridine. Heat was generated and a clear solution resulted. The mixture was allowed to stand with frequent shaking for three hours. At the end of this time it was poured slowly with stirring into two volumes of water. An oil settled out and solidified. The solid was filtered off and washed with water. The was either deacetylated immediately or purified by the following procedure. The crystals were dissolved by gentle heat in 2*N* sodium carbonate to separate them from any unchanged *p*-fluoroaniline. The alkaline solution was then acidified with 2*N* hydrochloric acid and the reprecipitated $N^1$-*p*-fluorophenyl-$N^4$-acetylsulfanilamide collected on a filter. Recrystallization from dilute ethanol produced an average yield of 80% of white crystals of $N^1$-*p*-fluorophenyl-$N^4$-acetyl sulfanilamide, melting at 190° C.

*VI. $N^1$-p-Fluorophenylsulfanilamide.*—The hydrolysis of the $N^1$-*p*-fluorophenyl-$N^4$-acetylsulfanilamide was carried out by essentially the same method as that used by Gelmo (11), modified by the addition of ethanol to dissolve the acetyl derivative. Suter (2) carried out this hydrolysis by refluxing with sodium hydroxide and obtained $N^1$-*p*-fluorophenylsulfanilamide in 47% yields and melting at 163–164° C.

A mixture of 15.5 Gm. (0.02 mol) of $N^1$-*p*-fluorophenyl-$N^4$-acetylsulfanilamide, 100 cc. of hydrochloric acid (sp. gr. 1.19), 225 cc. of ethanol and 50 cc. of water was placed in a 1-l. round-bottomed flask fitted with a reflux condenser. Heat was generated and a clear solution resulted. The mixture was refluxed for two hours, at the end of which time it was neutralized to litmus with sodium carbonate solution. The solid was collected on a Büchner funnel and washed free of carbonate with water. The solid was then dissolved in sufficient hot 2% aqueous sodium hydroxide, the solution diluted to 200 cc. with water and boiled with charcoal for two hours. The hot solution was carefully filtered and the treatment was repeated until the filtrate was only slightly yellow. Three volumes of water were added and sufficient 2% hydrochloric acid was slowly stirred into the mixture to make it neutral to litmus. The solid was filtered off, washed and recrystallized from 30% ethanol.

The $N^1$-*p*-fluorophenylsulfanilamide was obtained in flat white needles which sintered at 162–165° C., softened at 165° C., melted at 166.5° C.

(cor.). Sulfanilamide derivatives, in general, have been found to soften before they melt (1). The average yield is 55% of the theoretical based on the weight of *p*-fluoroaniline taken.

*Analysis.* Theor. for $C_{12}H_{11}O_2N_2SF$: 10.53% N. Found: 10.59% N.

## TOXICITY

The procedure followed in measuring the relative toxicities of sulfanilamide, $N^1$-phenylsulfanilamide and $N^1$-*p*-fluorophenylsulfanilamide was based in part on the work done by Rosenthal (12). The drug was suspended in olive oil by trituration in a mortar. Dilutions were made such that approximately 0.5 cc. of an olive oil suspension would be injected into each mouse, depending on the body weight. It was, therefore, necessary to employ 1% to 20% suspensions. Both sexes of mice weighing from 18 Gm. to 22 Gm. were used. These were deprived of food about twelve hours prior to injection. The injections were made intraperitoneally and sealed in with collodion. After the injection, the animals were observed hourly for twelve hours. A record was made of the symptoms, the time of death and the autopsy findings.

Long (13) describes the symptoms of sulfanilamide poisoning as those of a bilateral vestibular dysfunction and spastic paralysis. The symptoms of poisoning with $N^1$-*p*-fluorophenylsulfanilamide resembled in character those of sulfanilamide. However, the transition from one phase of the syndrome to the next is accomplished in a shorter time in sulfanilamide poisoning and the symptoms are more pronounced. The first manifestation of poisoning is a weakness in the hind legs which is followed by ataxia. The movements on stimulation become convulsive and incoördinated. In many cases there is a tendency to symmetrical convulsions with the occasional appearance of opisthotonus. This continues through a stage of prostration during which the animal is more difficult to arouse and which in most cases is terminated by death.

The autopsy shows some significant differences between the action of sulfanilamide and $N^1$-*p*-fluorophenylsulfanilamide. The bladder of a sulfanilamide-poisoned animal is generally hard, small and filled with a thick white mass, while that of an animal poisoned with $N^1$-*p*-fluorophenylsulfanilamide is often distended with a clear yellow liquid. The heart in either case may be normal or slightly dilated. The lungs are more profusely hemorrhagic in the sulfanilamide-poisoned animal.

Table I.—Toxicity of Sulfanilamide by a Single Intraperitoneal Injection in Mice

| Expt. No. | No. of Mice | Concn. of Suspn., % | Dose (Gm. per Kg.) | Percentage Mortality 12 Hr. | 30 Hr. | 46 Hr. | 48 Hr. | 54 Hr. |
|---|---|---|---|---|---|---|---|---|
| 5 | 4 | 7 | 1.730 | 0 | 25 | 75 | 75 | 100 |
| | 4 | 9 | 2.280 | 0 | 50 | 75 | 100 | 100 |
| | 4 | 12 | 3.010 | 75 | 100 | 100 | 100 | 100 |
| | 4 | 16 | 3.960 | 100 | 100 | 100 | 100 | 100 |
| 9 | 4 | 5 | 1.225 | 0 | 0 | 0 | 0 | 0 |
| | 4 | 7 | 1.614 | 0 | 0 | 25 | 25 | 25 |
| | 4 | 9 | 2.133 | 0 | 0 | 25 | 25 | 75 |
| | 4 | 11 | 2.805 | 25 | 50 | 75 | 75 | 75 |
| | 4 | 15 | 3.698 | 100 | 100 | 100 | 100 | 100 |

Table II.—Toxicity of $N^1$-*p*-Fluorophenylsulfanilamide by a Single Intraperitoneal Injection in Mice

| Expt. No. | No. of Mice | Concn. of Suspn., % | Dose (Gm. per Kg.) | Percentage Mortality 12 Hr. | 30 Hr. | 46 Hr. | 48 Hr. | 54 Hr. |
|---|---|---|---|---|---|---|---|---|
| 5′ | 4 | 2 | 0.450 | 0 | 0 | 25 | 25 | 25 |
| | 4 | 2.5 | 0.593 | 0 | 0 | 25 | 25 | 50 |
| | 4 | 3 | 0.782 | 0 | 0 | 25 | 75 | 75 |
| | 4 | 4 | 1.030 | 0 | 0 | 50 | 100 | 100 |
| | 4 | 6 | 1.359 | 0 | 0 | 100 | 100 | 100 |
| 6 | 3 | 6 | 1.413 | 0 | 33 | 100 | | |
| | 3 | 8 | 1.995 | 0 | 33 | 100 | | |
| | 3 | 12 | 2.818 | 0 | 66 | 100 | | |
| | 3 | 19 | 3.981 | 0 | 100 | 100 | | |
| 8 | 4 | 5 | 1.189 | 0 | 25 | 75 | 75 | 100 |
| | 4 | 7 | 1.679 | 0 | 25 | 100 | 100 | 100 |
| | 4 | 10 | 2.371 | 0 | 50 | 100 | 100 | 100 |
| | 4 | 14 | 3.350 | 0 | 100 | 100 | 100 | 100 |
| | 4 | 19 | 4.732 | 0 | 100 | 100 | 100 | 100 |
| 10 | 8 | 2 | 0.540 | 0 | 0 | 0 | 0 | 0 |
| | 6 | 3 | 0.760 | 0 | 17 | 50 | 50 | 50 |
| | 6 | 5 | 1.070 | 0 | 17 | 100 | 100 | 100 |
| | 6 | 6 | 1.520 | 0 | 33 | 100 | 100 | 100 |
| | 8 | 8 | 2.140 | 0 | 75 | 100 | 100 | 100 |

The preliminary experiments indicated that the action of $N^1$-*p*-fluorophenylsulfanilamide is delayed, and that it would be technically impossible to determine the $LD_{50}$ for a twelve-hour interval because of the concentration of the suspension it would be necessary to inject. It was also noted that the $N^1$-phenylsulfanilamide is still less toxic than the fluorinated compound in the twelve-hour interval. In order to compare these two substances, a 50% suspension of each in olive oil was prepared and an

amount of each corresponding to equal molecular quantities and representing the largest single dose that could be injected in 0.5 cc. volume was administered intraperitoneally to groups of eight mice. The doses employed amounted to 12.5 Gm. of $N^1$-*p*-fluorophenylsulfanilamide and 11.75 Gm. of $N^1$-phenylsulfanilamide per Kg. of body weight. Three out of eight mice receiving the fluorinated compound died in twelve hours and the remainder died in twenty-four hours. All the mice receiving the unfluorinated compound survived permanently with the exception of one which was found decapitated. From this experiment, it can be concluded that at the end of twelve hours both of these compounds are much less toxic than sulfanilamide and that the fluorinated compound is more toxic than the unfluorinated. The experiment further showed that under the experimental conditions employed, $N^1$-phenylsulfanilamide is so nontoxic that it would be impossible to determine an acute toxic dose given by single injection.

Experiments were then conducted to determine the $LD_{50}$ of sulfanilamide and $N^1$-*p*-fluorophenylsulfanilamide at different time intervals. Some of the conditions of these experiments and the results obtained are given in Tables I and II. From the results the $LD_{50}$'s at different time intervals were calculated by Kärber's method (14) and are recorded in Table III. It will be noted that the

Table III.—Comparison of the $LD_{50}$'s, as calculated by Kärber's Method, for Sulfanilamide and $N^1$-*p*-Fluorophenylsulfanilamide

| Expt. No. | Date | Time of Observation (Hours) | No. of Animals | $LD_{50}$ (Gm. per Kg.) Sulfanilamide | $LD_{50}$ (Gm. per Kg.) Fluorine Derivative |
|---|---|---|---|---|---|
| 5 | 2/25/40 | 11 | 16 | 3.57 | .. |
| 9 | 3/16/40 | 30 | 20 | 2.78 | .. |
| 6 | 3/2/40 | 30 | 16 | .. | 2.98 |
| 8 | 3/16/40 | 30 | 20 | .. | 2.82 |
| 5′ | 2/25/40 | 46 | 20 | .. | 1.07 |
| 9 | 3/16/40 | 48 | 20 | 2.90 | .. |
| 10 | 4/21/40 | 48 | 34 | .. | 0.98 |
| 9 | 3/16/40 | 54 | 20 | 2.48 | .. |
| 5′ | 2/25/40 | 54 | 20 | .. | 0.79 |

$LD_{50}$ of sulfanilamide is practically constant for a thirty, forty-eight or fifty-four hours' observation period. While the $LD_{50}$ of $N^1$-*p*-fluorophenylsulfanilamide is practically the same as that of sulfanilamide at the end of thirty hours, it drops to one-third at the end of fifty-four hours. The absolute values for the $LD_{50}$'s presented in the tables have large standard deviations and must be repeated employing more animals in order to determine accurately the significance of the observed differences. Several of the experiments in the tables were repeated in the summer when it was found that the relative $LD_{50}$'s remained the same but the absolute values changed slightly.

## SUMMARY

1. $N^1$-*p*-fluorophenylsulfanilamide has been prepared and characterized. The product was obtained in purer state and better yield than that obtained by the method reported by Suter (2).

2. The acute toxicity of a single intraperitoneal injection in an olive oil suspension of the $N^1$-*p*-fluorophenylsulfanilamide has been compared to that of $N^1$-phenylsulfanilamide and sulfanilamide. The $N^1$-phenylsulfanilamide was found to be practically non-toxic by a single dose. The toxicity of sulfanilamide and $N^1$-*p*-fluorophenylsulfanilamide is essentially the same at the end of thirty hours, while at the end of fifty-four hours the fluorinated compound has a delayed toxicity of about three times that of sulfanilamide.

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Sir William Ramsay (1852–1916) was awarded the Nobel Prize for Chemistry in 1902 in recognition of his discovery of the individual gaseous basic materials in the atmosphere and the determination of their place in the periodic system.