The Fate of Sulfuryl Fluoride in Wheat Flour

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The chemical fate of sulfuryl fluoride absorbed by graham flour has been studied. Graham flour was exposed to sulfuryl-S^{35} fluoride and, after suitable fractionation, the protein fraction was found to be responsible for almost all of the decomposition of the absorbed fumigant. Some sulfate was formed. Fluoride, as a consequence of the decomposition of sulfuryl fluoride, is likewise present.

SULFURYL FLUORIDE, a new fumigant found to have outstanding value for the control of structural and commodity insect pests, has undergone extensive study in the laboratory (*6*, *11*, *14*). Utilization of this fumigant is closely allied with the presence of foodstuffs, directly in the case of grain fumigation and indirectly in household fumigation.

Fumigation of various foodstuffs with sulfuryl-S^{35} fluoride results in a small amount of radioactive residue (*11*). Foodstuffs of moderate-to-high protein and fat content were implicated; fat in itself did not hold any residue but speculation would suggest that it acted as a solvent for reaction with the protein.

This paper describes a study of the fate of sulfuryl-S^{35} fluoride in graham flour.

Materials and Methods

Graham flour, which contains both the bran and the germ of wheat, was chosen to investigate the fate of sulfuryl fluoride; the flour seemed to be ideally constituted [fat, 2.2%; protein, 13.3%; and carbohydrate, 71.4% (*9*)] to act as a representative foodstuff for the absorption of measurable quantities of the fumigant.

Fumigation of Graham Flour with Sulfuryl-S^{35} Fluoride. Thirty milligrams of sulfuryl-S^{35} fluoride were transferred in vacuo to a fumigation vessel consisting of a 500-ml. round-bottomed flask connected to a vacuum manifold by means of an adaptor carrying a side arm. The side arm was so arranged that it could be immersed in a cooling bath. The fumigation vessel contained 10 grams of graham flour. After introduction of the gas, the evacuated vessel was not returned to atmospheric pressure but was sealed off by means of a stopcock and allowed to stand 92 hours at room temperature. The concentration of gas in this vessel was equivalent to 2 pounds per 1000 cubic feet. At the end of this time, the vessel was vented to the atmosphere, and the flour was removed and aerated in a hood to a constant count rate. The fumigation was carried out in this manner to maximize the extent and rate of fumigant penetration and sorption. Since the point of interest was qualitative in nature, it was felt, in this case, that the mode of fumigation would not alter the findings relative to normal fumigation practices. Figure 1 shows the fractionation scheme used in the subsequent treatment of the fumigated flour.

Extraction of Fumigated Flour. The fumigated flour was continuously extracted for 1 hour with 80% ethanol and then the residue was air-dried. This procedure removed 76% of the radioactive residue from the flour; no additional radioactivity was removed by a subsequent 7-hour continuous extraction with the same solvent mixture.

All counting was done in dish planchets at infinite thickness using a thin (<1.9 mg. per sq. cm.) end-window Geiger-Muller tube. Paper chromatograms were counted in increments by means of a special aluminum slide made to fit a Tracerlab SC9D shielding unit; the arrangement presented good geometry to the GM tube.

Treatment of Flour Extract with Dowex 50. The ethanol from the extraction of the fumigated flour was removed by distillation in vacuo. The resultant water mixture was passed through a 1.8-cm. column containing 20 grams dry weight of Dowex 50 ion exchange resin (acid form, 100–200 mesh). The water eluate and washings

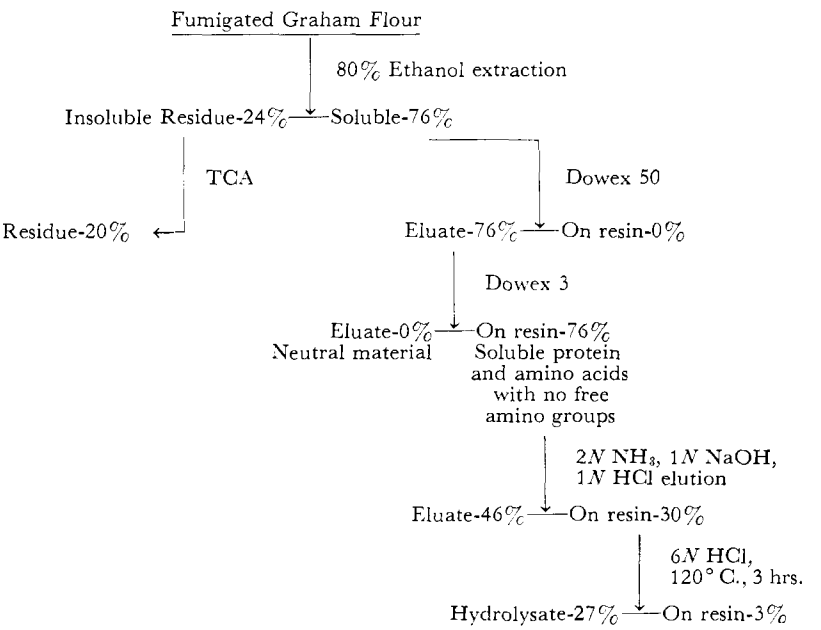

Figure 1. Fractionation procedure carried out on graham flour fumigated with sulfuryl-S^{35} fluoride

The per cent figures indicate the proportion of the total radioactivity initially present in the fumigated flour

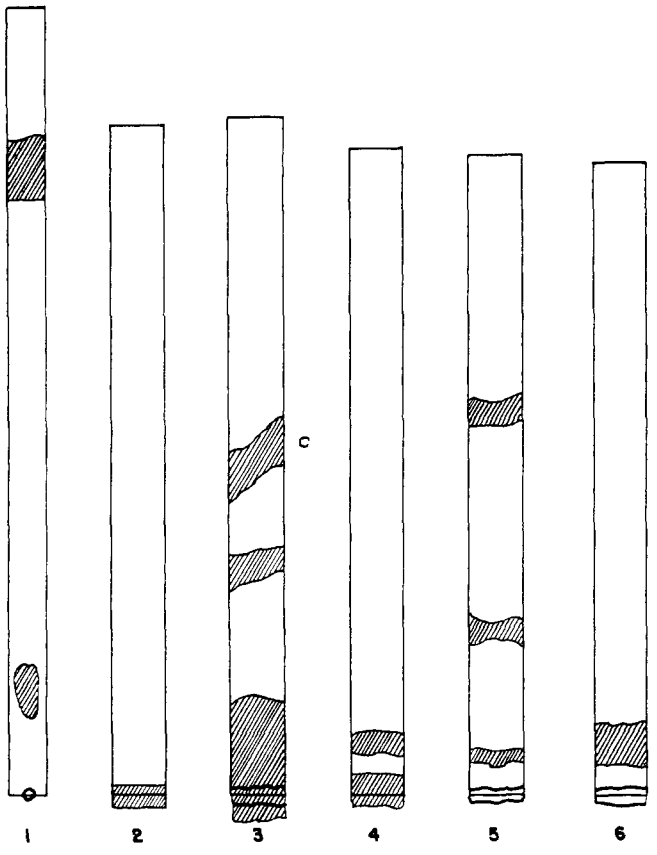

Figure 2. Autoradiographs of chromatograms from the 2*N* ammonia eluate of Dowex 3 and of sulfate-S^{35}

(1) n-Butanol–1.5*N* ammonia (1 : 1, v. : v.)
(2) Same solvent as 1, substrate was sulfate-S^{35}
(3) Ethanol–1.5*N* NH_3–water (90 : 5 : 5, v. : v.)
(4) Same solvent as 3, substrate was sulfate-S^{35}
(5) Phenol–H_2O (4 : 1, w. : w.)
(6) Same solvent as 5, substrate was sulfate-S^{35}

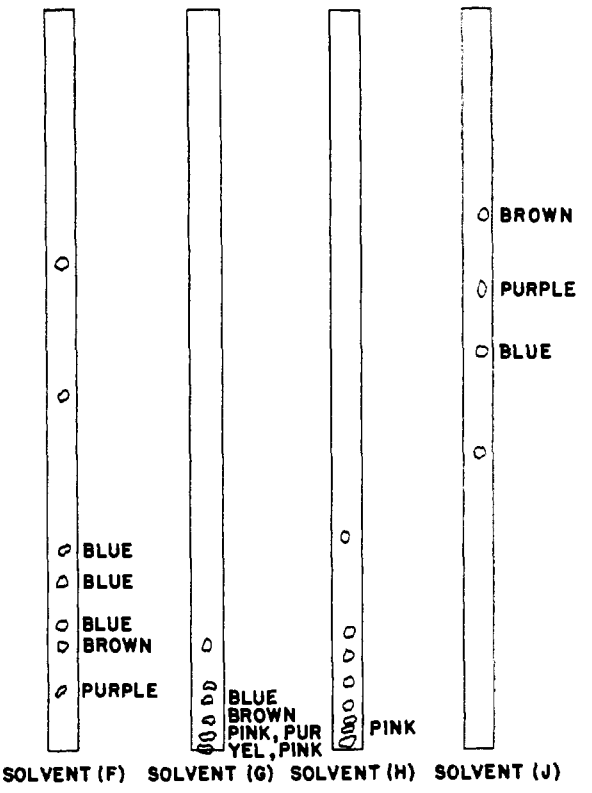

Figure 3. Chromatograms of the hydrolysate of the radioactive spot (C) from strip No. 3 of Figure 2

(F) N-Butanol–propionic acid–water (7 : 3 : 10, v. : v.)
(G) 2,6-Lutidine–ethanol–water (55 : 25 : 20, v. : v.), with 2 ml. of diethylamine added to every 100 ml. of the mixture.
(H) Methyl ethyl ketone–propionic acid–water (6 : 2 : 2, v. : v)
(J) 2-Methoxyethanol–propionic acid–water (6 : 2 : 2, v. : v.)

were then combined. There was no radioactive residue deposited on the resin after this treatment.

Treatment of Flour Extract with Dowex 3. The eluate and combined washings from the treatment with Dowex 50 were put through a 1.8-cm. column containing 20 grams dry weight of Dowex 3 resin (free-base form, 100–200 mesh). The water eluate from this column contained no radioactive material; the radioactive residue had been quantitatively transferred to the Dowex 3 resin.

Elution of Dowex 3 Resin. The Dowex 3 resin, which contained all the radioactivity from the fumigated flour extracts, was eluted with 2*N* ammonia to remove 61% of the radioactive material. Attempts to elute the radioactivity residual to the resin by using 1*N* sodium hydroxide or 1*N* hydrochloric acid failed.

Investigation of Residual Activity on the Dowex 3 Resin. A portion of the eluted Dowex 3 resin and some fresh resin were each heated at 120° C. in sealed-tubes with 6*N* hydrochloric acid for 3 hours. At the end of this time, the tubes were cooled and opened, and the treated resins were thoroughly washed with water. This treatment removed 92% of the radioactive material residual to the resin.

To identify any amino acids that might have been removed from the treated Dowex 3 resin, the 6*N* hydrochloric acid hydrolysate from this resin was examined by means of buffered, one-dimensional filter paper chromatography according to the procedure described by McFarren (*10*). The results were then compared with those derived from the fresh resin hydrolysate.

Two solvents were used in this experiment: McFarren's solvents *A* and *B* (*10*). Known amino acids, the radioactive hydrolysate, and the hydrolysate from the fresh resin were run in these two solvents. The paper in the chamber containing solvent *A* was allowed to equilibrate 5.5 hours, and that to be developed in solvent *B*, 2 hours. The fresh resin hydrolysate gave no ninhydrin-positive spots. The hydrolysate from the radioactive resin showed two ninhydrin-positive spots in solvent *A* and 3 positive spots in solvent *B*.

Investigation of Radioactive Substances in 2*N* Ammonia Eluate of Dowex 3 Resin. The 2*N* ammonia eluate of the Dowex 3 resin was concentrated in vacuo and redissolved in water. After first putting this solution through a column of Dowex 50 (acid form, 100–200 mesh), on which there was no retention of radioactivity, and again concentrating in vacuo, the solution was developed descending in 3 solvents to give the chromatograms shown in Figure 2. Unwashed Whatman No. 1 paper was used, and the papers were equilibrated with the solvents for 3 hours before development. In addition, sulfate-S^{35} was run simultaneously in these three solvents.

This experiment shows that sulfate and at least two distinct radioactive substances are present in the 2*N* ammonia eluate from the Dowex 3 resin.

The radioactive spots displayed on the chromatograms in Figure 2 were eluted from the paper with concentrated aqueous ammonia and the eluate was evaporated to dryness in vacuo. These samples were heated at 120° C. (autoclave) in 3*N* hydrochloric acid for 16 hours in sealed tubes. The hydrochloric acid was removed by distillation in vacuo and then by heating 1 hour in a vacuum oven (water aspirator) at 100° C. The residues were dissolved in 0.1 ml. of 10% isopropanol and developed on Whatman No. 1 paper (unwashed),

descending, at room temperature. Four solvents were used as described by Alexander and Elvehjem (*1*). The dried chromatograms were sprayed with ninhydrin solution as already described. Figure 3 is a map for each of the four solvents and shows the ninhydrin-positive spots, with color indicated, which represent the hydrolyzed, radioactive spot (*C*) from chromatogram No. 3, Figure 2. At least eight different amino acids are present. The remainder of the chromatograms, which are not included here, displayed an almost identical pattern of ninhydrin-positive spots.

Tracing Sulfate-S^{35} through Separation Scheme Used for Fumigated Flour. A water solution of sodium sulfate-S^{35} was thoroughly mixed with some graham flour in such proportion that the consistency of the flour was not changed. This mixture was submitted to the extraction and fractionation procedures described above for the fumigated flour. Seventy per cent of the sulfate-S^{35} was removed from the flour by extraction, none of the extracted radioactivity was retained by the Dowex 50 resin, and all of it was held up on the Dowex 3 resin. In addition, the sulfate-S^{35} retained by the Dowex 3 resin was easily and completely eluted by 2*N* ammonia.

Trichloroacetic Acid (TCA) Treatment of Fumigated, Extracted Flour. The sulfate-S^{35} remaining in the alcohol-extracted, flour-sulfate-S^{35} mixture was completely removed by shaking the flour mixture with a 5% solution of TCA for 1 hour at room temperature. Accordingly, the alcohol-extracted fumigated flour, which still contained 24% of its initial radioactivity, was subjected to the TCA extraction. As a result of this treatment, only a small amount of this radioactivity was removed from the flour. The flour still retained 20% of the initial radioactivity.

Reaction of Sulfuryl Fluoride with Water in Presence of Phosphate. Sulfuryl fluoride was bubbled through 20 ml. of 1*M* phosphate buffer at pH 6.5 for 1 hour at room temperature using a gas dispersion tube. This solution gave a very strong positive test for fluoride ion using a zirconium-alizarin reagent (*3*). There was no sulfate present; however, after heating an aliquot of this solution, to which had been added a few drops of 6*N* hydrochloric acid, for 10 minutes in the presence of barium ion, a precipitate of barium sulfate formed. After standing at room temperature for 4 days, the original reaction mixture also gave a precipitate of barium sulfate. The behavior expected of sulfuryl fluoride and water is initial formation of fluorosulfonic acid (the barium salt of which is water soluble), and then slow conversion of this to sulfate in acid medium. Passing sulfuryl fluoride through water for 3 hours at room temperature resulted in the formation of very little, if any, fluoride. It seems clear, then, that phosphate in some way serves to increase the rate of this reaction.

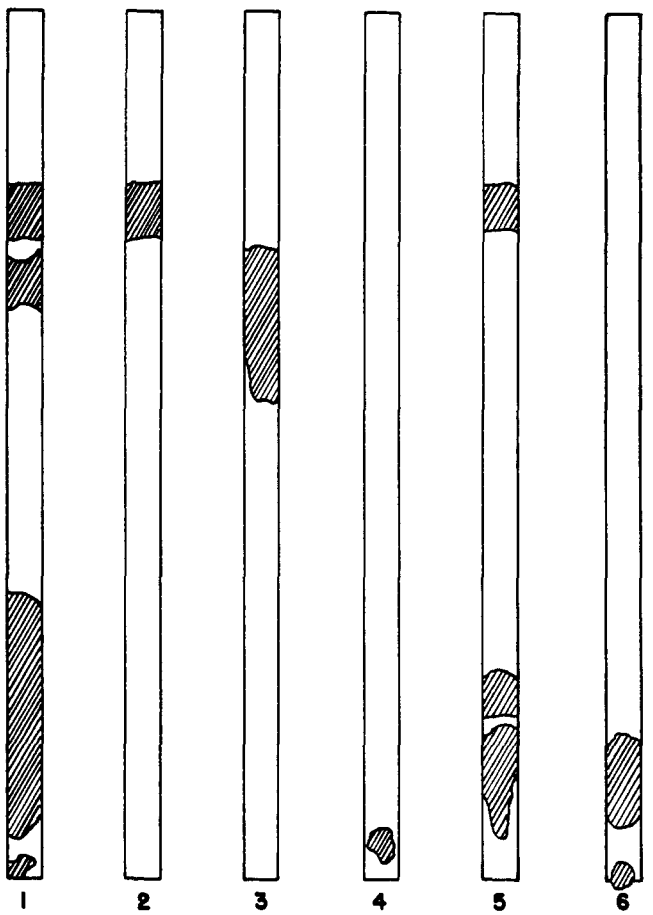

Figure 4. Chromatograms of the products from the reaction of sulfuryl fluoride with water in the presence of phosphate

The solvent system was methanol–1.5*N* NH_3–water (20:1:4, v.:v.)

(1) Reaction mixture
(2) Ammonium fluorosulfonate
(3) Ammonium fluoride
(4) Ammonium sulfate
(5) Phosphate buffer and ammonium fluorosulfonate
(6) Phosphate buffer

The reaction mixture (sulfuryl fluoride and phosphate buffer), ammonium fluorosulfonate [prepared according to Traube *et al.* (*15*)], ammonium fluoride, ammonium sulfate, a mixture of phosphate buffer (pH 6.5) and ammonium fluorosulfonate, and phosphate buffer (pH 6.5) were chromatographed, descending, at room temperature, on Whatman No. 1 paper (washed in 1% oxalic acid). The solvent system was methanol–concentrated aqueous ammonia–water (20:1:4); the paper was equilibrated 2 hours before developing. To detect the components, the bromphenol blue acid-base indicator solution described by Kennedy and Barker (*7*) was used.

The results of this experiment are shown in Figure 4. The reaction mixture, No. 1, shows spots for fluoride and ammonium fluorosulfonate as well as an extended phosphate spot which probably indicates some complex phosphates. These complex phosphate spots are also in evidence in the phosphate buffer–ammonium fluorosulfonate mixture, No. 5.

Discussion

Distribution of Radioactivity in Fumigated Graham Flour. As a result of successive fractionation steps applied to graham flour after fumigation with sulfuryl-S^{35} fluoride, the radioactivity was found to be distributed as shown in Figure 1. The extractable radioactivity has settled 100% on the Dowex 3 anion exchange resin and must, therefore, reside in molecules which are anionic in character–i.e., carboxylic acids, sulfate, etc. This result is interpreted as indicating that the predominant reaction is one involving free amino groups of amino acids and protein. If there had been reaction with hydroxyl we would expect to find some radioactivity in the neutral fraction. This was not the case.

Figure 1 shows that the insoluble flour residue, after 80% ethanol extraction, retained 24% of the radioactivity. In addition, experiments with radioactive sulfate showed that the same extraction pattern resulted as with the fumigated flour. Subsequent treatment of the flour–sulfate-S^{35} residue with 5% aqueous trichloroacetic acid (TCA) solution completely dissolved the sulfate-S^{35} as the protein was precipitated. However, in the case of the fumigated extracted flour residue, the (TCA) treatment dissolved very little radioactivity—the activity was still retained in the precipitated protein fraction. Therefore, the activity remaining in the insoluble residue fraction, as shown in Figure 1, was not sulfate, but was in some way fixed to the protein fraction of the residue.

The affinity of the radioactivity for the Dowex 3 anion exchange resin suggests that the sulfuryl fluoride has reacted with free amino groups on amino acids and protein leaving carboxyl groups free to exchange with the resin. Radioactive sulfate could also be present. Elution of this resin with 2*N* ammonia resulted in removal of 61% of the residual radioactivity. Efforts to remove the remaining activity with other solvents failed. An apparent explanation for the relative intractability of this tightly bound activity could be one involving a reaction between *N*-fluorosulfonyl derivatives of amino acids and the free amino groups of the resin to form *N*-(amino acid or protein)-*N'*-(resin) sulfamides. Acid hydrolysis of the resin completely removed the radioactivity, and ninhydrin-positive substances, presumably amino acids, were found to be present in the hydrolysate. No attempt was made to identify these compounds. The important point here is not so much their identity, but the presence of ninhydrin-positive compounds after hydrolysis of the resin.

That portion of the radioactivity which was easily eluted from the anion exchange resin was subjected to paper chromatography in three different solvents. The activity was displayed in such a manner as to indicate radioactive sulfate and at least two other entities. Elution, hydrolysis, and subsequent chromatography of these spots revealed that each spot yielded at least eight amino acids. This result is interpreted to mean that each of these radioactive spots was soluble polypeptide. The amino acid content of each of these radioactive spots was nearly identical, qualitatively. The author believes that this result could only arise from polypeptide

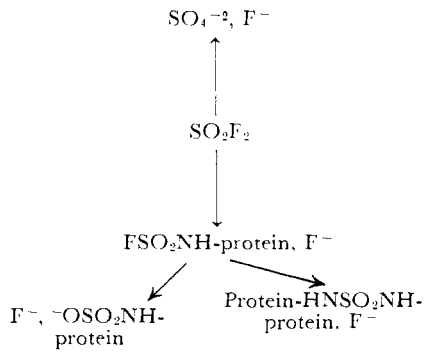

Figure 5. Proposed scheme for the breakdown of sulfuryl fluoride in graham flour

molecules where the qualitative amino acid content is not substantially varied but where differences might well be expected in their chromatographic behavior. In this respect, gliadin of wheat has been reported to be at least two, and perhaps more, electrophoretically distinct proteins (*12, 13*). It seems reasonable, then, that these fractions would also exhibit different chromatographic mobilities.

Sulfate is formed as a result of conventional hydrolysis of sulfuryl fluoride. This reaction proceeds stepwise, first to fluorosulfonic acid, then to sulfate, and it has been shown herein to be catalyzed by phosphate. This reaction might also be expected to take place in the environs of the flour, and, indeed, radioactive sulfate has been found in the eluate of the Dowex 3 anion exchange resin. In addition, radioactive sulfate traced through the fractionation scheme ended up exactly where it was detected in the sulfuryl-S^{35} fluoride experiments.

The amino acid content of gliadin (wheat) has been reported (*2*), to include 19 common amino acids. Of these, it seems reasonable to predict that only those contributing *N*-terminal amino groups to the protein molecules would offer a reaction site for sulfuryl fluoride. The *N*-terminal amino acids in gliadin protein have been investigated, and the consensus indicates that these are phenylalanine, histidine, and lysine (*4, 5, 8*).

In view of the work described here and the results reported on the *N*-terminal amino acid determinations in gliadin (wheat), the radioactivity residual to the graham flour after sulfuryl-S^{35} fluoride fumigation is felt to be present in the form of sulfate and as reaction products resulting from a chemical combination with the *N*-terminal amino nitrogen of protein and free amino acids, most likely with phenylalanine, histidine, and lysine. The products of this reaction will be the *N*-fluorosulfonyl derivatives, *N,N'*-disubstituted sulfamides, and *N*-substituted sulfamate. Moreover, the work described here strongly suggests that the reactivity of the *N*-fluorosulfonyl functional groups is sufficient to cause these derivatives to be ultimately converted in vivo to *N,N'*-disubstituted sulfamides or *N*-substituted sulfamic acids. Figure 5 shows the proposed scheme for the breakdown of sulfuryl fluoride in graham flour.

An additional product resulting from the above reactions is fluoride. The total fluoride residue in graham flour under normal fumigation procedures is about 15 p.p.m. (*11*). Fluoride residues in grain may be a potential health hazard insofar as the grain finds its way into our diets. The results of this investigation show that any grain fumigation using sulfuryl fluoride may have to contend with inorganic fluoride residues.

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