Analysis of Cottonseed for Residues of Tributyl Phosphorotrithioite

GEORGE R. BOYD Jr., and MARGARET ANNE BARBER
Virginia-Carolina Chemical Corp., Richmond, Va.

Tributyl phosphorotrithioite (the active ingredient in Folex) is a remarkably effective cotton defoliant. An analytical method for determining residues in cottonseed products is described. The method utilizes sodium borohydride to assist in the hydrolysis of the trithioite to liberate butyl mercaptan which is volatilized out of the reaction mixture, trapped in mercuric acetate solution, and determined colorimetrically. The analytical procedure can detect 0.05 p.p.m. of the trithioite using a 100-gram sample, and typical analyses are given for seed from cotton defoliated with Folex. This hydrolytic technique is new and should be applicable to the analysis of a variety of sulfur-containing phosphorus esters.

THE PHYSIOLOGICAL effects of phosphorus esters upon insects and animals have been studied intensively for several years and their mode of action is generally well understood, but comparatively little is known of their effect upon living plants. Even less is known of the activity of analogous sulfur-containing esters, the phosphorothioites and phosphorothioates. Therefore, it was somewhat of a surprise when it was discovered (*3*) that very small amounts of some of the trialkyl phosphorotrithioites would defoliate certain plants without killing the plant or desiccating the leaves. The commercial outgrowth of this discovery is tributyl phosphorotrithioite, $(n\text{-}C_4H_9S)_3P$. This compound is known as merphos. It is formulated as a 75% emulsifiable concentrate or as a 5% dust and sold under the trade name of Folex.

The most important application of Folex is in the defoliation of cotton where, under usual conditions, 1 or 2 pounds per acre of active ingredient will give complete defoliation in from 5 to 7 days (*1*).

Since cottonseed is the starting material for food for both human and animal consumption, analytical methods suitable for product assay and residue determinations of this material were required.

The chemistry of the thiol esters of the phosphorus acids is almost as unexplored as their physiological effects. Very little information has been published on reactions which would be suitable as bases for the specific analytical determination of merphos. The most promising approach seemed to lie in the decomposition of merphos to form butyl mercaptan which could be separated by volatilization and determined by any one of several attractive methods.

An early analytical procedure was based on a scheme in which merphos was hydrolyzed in a two-stage process by boiling first with sodium hydroxide and then with hydrobromic acid. The extent of hydrolysis obtained in this manner, however, was not satisfactorily reproducible and variation among replicate determinations seriously limited the sensitivity of the method.

The analytical methods finally evolved are based upon the observation that sodium borohydride will decompose both tributyl phosphorotrithioite and its oxidation product, *S,S,S*-tributyl phosphorotrithioate, liberating the theoretical amount of butyl mercaptan. Decomposition is quantitative under the specified conditions and results of a high degree of precision can be obtained. The mercaptan is volatilized from the reaction mixture and trapped in a suitable absorbing solution. It can then be determined volumetrically or colorimetrically depending on whether macro or micro quantities of merphos are expected.

Only the micromethod and its application to the determination of residues of merphos in cottonseed products are discussed in this article.

Although complete removal of mercaptans from aqueous solutions is easily accomplished by distillation, recovery from solutions containing organic solvents is much more difficult (*4*). For this reason most of the oily extractives of cottonseed must be removed before distillation of the mercaptan. Cleanup of the cottonseed extract using partition between hexane and acetonitrile or between hexane and dimethylformamide was investigated. Neither of these solvent systems gave quantitative recovery of merphos. A variety of solid absorbents were tested and of these only Florisil gave quantitative recovery of merphos added to hexane extracts of cottonseed.

Reagents

Amine Solution. Dissolve 5 grams of *N,N*-dimethyl-*p*-phenylenediamine hydrochloride in 1 liter of concentrated HCl.

Ferric Chloride Solution. Dissolve 67.6 grams of $FeCl_3.6H_2O$ in 500 ml. of water and 72 ml. of boiled concentrated HNO_3. Dilute to 1 liter with water.

Mercuric Acetate. Dissolve 50 grams of mercuric acetate in 500 ml. of water containing 50 ml. of CH_3CO_2H. Dilute to 1 liter.

Hydrochloric Acid. Dilute 100 ml. of concentrated reagent with 200 ml. of water.

Sodium Hydroxide. Approximately 0.5*N*.

Sodium Borohydride. Solid.

Nitrogen Gas. Cylinder gas passed through alkaline pyrogallol to remove traces of oxygen.

Florisil. Mixture (1 to 1) of 60 to 100 mesh and 100 to 200 mesh. Three batches of commercial Florisil, used without preliminary treatment, gave equally low blanks and good recovery of added merphos.

Hexane. Technical grade from Esso Standard Division, Humble Oil and Refining Co., is satisfactory.

Apparatus

Coffee-mill type food grinder.

Soxhlet extractor for 43-mm. thimbles.

Spectrophotometer with 20-mm. cells.

Hydrolysis and evolution apparatus. It consisted of a 250-ml. reaction flask fitted with a nitrogen gas inlet tube. This is connected to a two-way adapter

leading to a 30-ml. dropping funnel, vented with nitrogen, and a condenser. The top of the condenser is connected by a U-shaped gas delivery tube to a 25-ml. blood sugar tube which serves as a collector.

Procedure

Extraction. Select 100 grams of cottonseed which is representative of the entire sample and pass through a coffee-mill type grinder to break open the hulls. Divide this sample into two approximately equal portions and pack each into a 43-mm. extraction thimble. Place these in Soxhlet extractors and extract with 200 ml. of hexane for 4 hours. Evaporate the extracts obtained from the two half-samples on a steam bath in a stream of filtered air. Combine the extracts into a single flask and evaporate to a total volume of about 125 ml.

Cleanup of Extracts. Prepare a column of 15 grams of Florisil in a Jones reductor tube, 20 mm. in diameter. Pour the concentrated extract onto this column. When the level of the extract has reached the top of the filling, add 50 ml. of hexane to the flask. Rinse the flask and pour onto the column, washing the walls of the upper part of the tube in so doing. Allow the wash solution to pass through the column until the upper level of the hexane has reached the Florisil. Discard all effluent collected to this point. Place a 250-ml. flat-bottomed balloon flask below the outlet of the tube. Add 100 ml. of acetonitrile to the top of the column and allow to drain completely. Place the collecting flask on the steam bath and evaporate essentially to dryness in a stream of filtered air.

Hydrolysis of Merphos. Place the flask containing the residue on the reduction apparatus and flush with nitrogen for 10 minutes. Pour 10 ml. of mercuric acetate solution into the 25-ml. blood-sugar tube. Place this in the apparatus and insert the delivery tube almost to the bottom of the tube. Adjust the rate of nitrogen flow to deliver a continuous stream of bubbles through the trap. Maintain this flow of nitrogen throughout the remainder of the determination. Remove the flask containing the residue, add 25 ml. of 2-propanol, 25 ml. of sodium hydroxide solution, and about 0.25 gram of solid sodium borohydride. Swirl to mix and replace on the apparatus. Heat to boiling and allow to reflux for 30 minutes. Through the addition tube, add 25 ml. of hydrochloric acid solution dropwise. Follow this with 25 ml. of water also added very slowly. Continue heating and sweeping with nitrogen for 1 hour.

Determination of Butyl Mercaptan (*5*). Remove the trap tube and rinse the inlet tube twice with small amounts of water. Add 1.5 ml. of dimethyl-*p*-phenylenediamine solution. Stopper the tube with a clean rubber stopper and shake well. Add 0.5 ml. of ferric chloride solution, dilute to the mark with water, stopper, and mix well. Allow the tube to stand 30 minutes and then read the light transmission in a 2-cm. cell at a wave length of 500 mμ. Use a reagent blank to set the spectrophotometer on 100% transmission.

Prepare a calibration curve by carrying solutions containing 25, 50, 100, 200, and 300 μg. of merphos through the reduction and color-forming stages of the procedure. A plot of transmittance *vs.* weight of merphos is a straight line for this range of concentration.

Table I. Recovery of Merphos Added to Untreated Cottonseed

(DPL-15 and Deltapine TPSA varieties; 1959 season)

Added, μg.	Range Found, μg.	Number of Replicates	% Recovery	Mean % Recovery
98	90–99	6	92–101	98
49	41–52	2	84–105	94
25	18	1	72	72
10	6–13	4	60–130	97
5	2–4	3	40–80	60
Mean recovery				88

Table II. Residues of Merphos on Seed from Cotton Defoliated with Folex and from Controls

Rate of Application, Pints/Acre	Total Apparent Residue, P.P.M.	Actual Merphos Residue, P.P.M.
Folex in Water		
0[a]	0.10	...
0	0.09	...
0	0.09	...
0	0.10	...
0	0.10	...
0	0.09	...
2	0.09	0.00[b]
2	0.11	0.01
2	0.28	0.18
2	0.16	0.06
2	0.16	0.06
2	0.13	0.03
2	0.12	0.02
2	0.29	0.19
0[c]	0.09	...
0	0.11	...
0	0.10	...
0	0.09	...
1	0.15	0.05[b]
1	0.11	0.01
1	0.16	0.06
1	0.11	0.01
2	0.20	0.10
2	0.21	0.11
2	0.16	0.06
2	0.12	0.02
0[d]	0.11	...
0	0.06	...
0	0.07	...
2	0.19	0.11[e]
2	0.19	0.11
2	0.20	0.12
2	0.20	0.12
2	0.19	0.11
2	0.14	0.06
2	0.08	0.00
Folex in Diesel Oil		
2[a]	0.15	0.05[b]
2	0.19	0.09
2	0.22	0.12
2	0.22	0.12
2	0.29	0.19
2	0.27	0.17
2[f]	0.22	0.14[e]
2	0.24	0.16
2	0.20	0.12
2	0.21	0.13
2	0.21	0.13
2	0.27	0.19
Folex Dust, 5%		
0[g]	0.12	...
0	0.09	...
0	0.14[d]	...
0	0.08	...
0	0.04	...
Pounds/Acre		
20	0.20	0.10[h]
20	0.28	0.18
20	0.17	0.07
20	0.19	0.09
20	0.23	0.13
	0.18	0.04[h]
20[i]	0.13	0.00
20	0.20	0.06

[a] Experiments in this column were done at College Station, Tex. [b] Mean blank value, 0.10 p.p.m. subtracted from total apparent residue to find actual merphos residue. [c] Experiments in this column were done at Temple, Tex. [d] Experiments in this column were done at El Paso, Tex. [e] Mean blank value, 0.08 p.p.m. subtracted from total apparent residue to find actual merphos residue. [f] Experiments in this column were done at Isletta, Tex. [g] Experiments in this column were done at Fort Valley, Ga. [h] Mean blank value, 0.14 p.p.m. subtracted from total apparent residue to find actual merphos residue. [i] Experiments in this column were done at Shafter, Calif.

Evaluation of the Method

Even with the cleanup procedure employed and the additional purification effected by distillation of the butyl mercaptan, there is a detectable amount of mercaptan-like material released from extracts of seed from cotton which has not been treated with merphos. This varies with the location and variety of the cotton, but is usually small and quite reproducible for samples of similar background. This "blank value" must be subtracted from the total apparent merphos found to give the true residue. To obtain a measure of precision of the analytical procedure applied to untreated seed, six samples of undefoliated DPL-15 cotton were analyzed. The results were in the range of 0.08 to 0.11 p.p.m., the mean value being 0.101 and the mean deviation 0.008 p.p.m.

To determine the accuracy of the method, known amounts of merphos were added to samples of seed from undefoliated cotton and these were extracted and analyzed as described. The results are shown in Table I. Data indicate that good recovery of added merphos can be obtained in the range of from 5 to 100 μg. This corresponds to a concentration range of 0.05 to 1.0 p.p.m. based upon the 100-gram sample of cottonseed recommended.

Application of the Method

This analytical procedure has been applied to several hundred samples of seed from cotton defoliated with Folex under a wide variety of conditions. Table II shows typical results obtained on seed from cotton defoliated by Folex applied with water, Folex with diesel oil, and as a 5% Folex dust. In obtaining these data, every effort was made to analyze the cottonseed as soon as possible after harvest. However, because of the handling and shipping operations involved, periods of from 5 to 12 weeks elapsed between the application of Folex and the analysis of the seed described in Table II.

Discussion

Eggertsen and Weiss (*2*) reported that lithium aluminum hydride reduces phosphate esters completely to phosphine, the alkoxyl groups presumably forming alcohols. At the start of this investigation, it was found that lithium aluminum hydride or sodium borohydride would liberate butyl mercaptan quantitatively from tributyl phosphorotrithioite. Because of the ease of handling, sodium borohydride was chosen for routine use. Surprisingly, this reagent does not reduce the phosphorus in the thiolesters to phosphine.

If the reaction is carried out using *S,S,S*-tributyl phosphorotrithioite, all of the phosphorus is found to be present as phosphorus acid. If the reaction is carried out using *S,S,S*-tributyl phosphorotrithioate or tributyl phosphorotetrathioate, the phosphorus is recovered as phosphoric or thiophosphoric acid. In the case of the tetrathioate no hydrogen sulfide is eliminated. This is in agreement with the findings of Eggertsen and Weiss, who reported that no hydrogen sulfide was evolved from parathion when this was reduced with lithium aluminum hydride.

Since the phosphorus present in merphos ultimately appears as an acid, the over-all reaction is effectively an hydrolysis rather than the reduction which might be predicted. This may be the result of the formation of an intermediate compound between the sodium borohydride and the thio compound which is subsequently hydrolyzed by aqueous hydrochloric acid forming phosphorous acid and butyl mercaptan; or it may be that sodium borohydride is simply a more effective hydrolyzing agent than sodium or potassium hydroxide. Whatever the explanation, it has been well established that more consistent results can be obtained using sodium borohydride than by using the more common bases.

This reaction with sodium borohydride has also been applied to the mixed oxygen-sulfur esters tributyl phosphorodithioite and tributyl phosphorothioite. In each case, the theoretical amount of mercaptan was recovered: two molecules from the dithioite and one from the monothioite.

Since undelinted cottonseed is the raw agricultural commodity whose analysis for residues of merphos was required, most of the analytical work was done on such samples. During the course of these analyses, it was noticed that there was a correlation between the time that elapsed between application of Folex and analysis of the seed and the residue found. This suggested that the residue is on the outer surface of the seed, probably as the result of mechanical contamination, rather than inside of the cottonseed as a result of translocation.

To test this conclusion, several samples of seed were completely delinted in a laboratory machine which allowed recovery of both lint and bare seeds. These fractions were analyzed separately and it was found that the lint contained all of the merphos and the seed contained none.

This analytical procedure can be applied to the determination of merphos residues in cotton foliage, lint, stems, seed hulls, and seed meats. It cannot be applied to the analysis of crude cottonseed oil unless more efficient cleanup steps are included.

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