±3% accuracy on duplicate injections of the same solution. This, too, will vary with instruments, operators, and operating conditions. Each laboratory will have to choose its own set of operating parameters. The method sensitivity using the activated carbon cleanup procedure was 0.2 p.p.m., while the Florisil cleanup allowed a sensitivity of 0.1 p.p.m. The difference in sensitivity resulted from a background of apparent halogen response which was partially removed by the Florisil treatment. The removal of an interfering DDT-type compound by selective elution from a Florisil column was necessary because both compounds (DDT and Dilan) had overlapping retention times. The Florisil procedure was effective and allowed good recovery of the Dilan.

A procedure, including cleanup, for the analysis of Dilan residues in pears was achieved. Evaluation of entomological data will undoubtedly be reported elsewhere.

Acknowledgment

The expert technical assistance of Nancy Nash is to be noted in preparing the samples and obtaining the residue data.

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PESTICIDE RESIDUES

Colorimetric Determination of 6-Methyl-2,3-quinoxalinedithiol Cyclic Carbonate (Morestan) Residues in Apples and Pears

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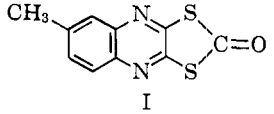
An analytical method for microdetermination of spray residues of Morestan is described. The procedure involves a hydrolysis with concentrated ammonium hydroxide to give 6-methyl-2,3-quinoxalinedithiol. Subsequent treatment with ammoniacal nickel reagent gives a red-colored chelate which is measured at 540 mμ. The method has been used for determination of Morestan residues in apples and pears.

MORESTAN (trademark, Farbenfabriken Bayer, A.G.) is 6 methyl-2,3-quinoxalinedithiol cyclic carbonate. The compound, formerly referred to as Bayer 36205, has been shown to be very effective in controlling several mite species on a wide range of crops. Control of both resistant and nonresistant strains has been reported for periods of up to 4 weeks. Morestan is also effective against pear psylla, white flies, and aphids, and has given excellent control of powdery mildew on a variety of crops.

The structure of Morestan is as follows:

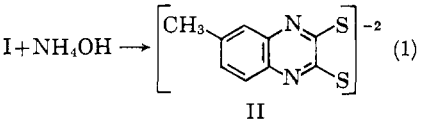


I

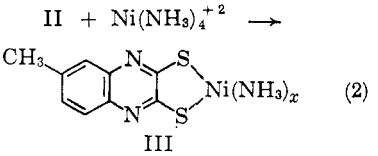
Little information appears in the literature regarding the chemical and physical properties of the compound. It is closely related to the insecticide Eradex, which is 2,3-quinoxalinedithiol cyclic trithiocarbonate.

Previous work in the authors' laboratory has shown that Eradex, upon treatment with ammonium hydroxide, is hydrolyzed to give 2,3-quinoxalinedithiol. The latter compound has been proposed by Morrison and Furst for the colorimetric determination of nickel (*7*). Morestan under the same conditions gives 6 methyl-2,3-quinoxalinedithiol which, as expected, also reacts with ammoniacal nickel to give a colored complex.

A partial equation for the reaction is as follows:



II



III

Structure III is analogous to the one postulated by Morrison and Furst (*7*) for the complex derived from 2,3-quinoxalinedithiol. Maximum absorbance of III is at 540 mμ. The color is stable and follows Beer's law in concentrations from 0 to 15 μg. per ml.

Morestan is relatively nonpolar, and can be quantitatively extracted from a 2:1 acetone:water solution into an equal volume of Skellysolve B. It is not strongly held by adsorbants, such as alumina, and can be easily eluted using nonpolar solvents. The compound is rapidly hydrolyzed in concentrated ammonia. A 10-minute hydrolysis and a 20-minute color development following addition of nickel reagent gave completely reproducible results and was adopted for routine use.

Samples are analyzed by blending with acetone, filtering, and extracting the filtrate with Skellysolve B. Morestan partitions into the Skellysolve B, and the bulk of the crop extractives remains in the aqueous acetone phase. This extraction provides considerable, though not sufficient, cleanup. Chromatography on alumina removes enough of the remaining interferences to permit

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hydrolysis and color development. In some cases, the final solution, following color development, is turbid and contains interfering plant pigments. These are removed by extracting with benzene. The interferences partition quantitatively into the benzene, and the colored complex remains in the lower aqueous phase.

Experimental

Apparatus. Blenders, Waring or equivalent, with 1-quart jars marked at 600-ml. level.

Chromatographic tubes, 2 × 40 cm., fitted with Ultramax stopcock.

Colorimeter, Evelyn.

Food Chopper, Hobart.

Reagents. Acetone, reagent, redistilled.

Alumina, acid washed (Merck).

Ammoniacal nickel solution. Dissolve 6 grams of reagent nickelous chloride hexahydrate in 50 ml. of distilled water. Add 50 ml. of concentrated ammonium hydroxide (reagent) and mix.

Benzene, technical, redistilled.

Blank solution. Mix 250 ml. of acetone, 175 ml. of concentrated ammonium hydroxide, 0.625 ml. of ammoniacal nickel solution together in a 1-liter separatory funnel. Add 75 ml. of benzene and shake to extract. Drain the aqueous layer into a 500-ml., ground-glass stoppered bottle.

Morestan stock solution. Dissolve 0.10 gram in 1 liter of acetone to give a 100 μg. per ml. solution. Store in the dark or in an amber bottle.

Skellysolve B, redistilled.

Sample Preparation. Chop the frozen sample with dry ice in a food chopper and hold overnight in frozen storage to allow the dry ice to sublime. Use approximately 2 pounds of dry ice per pound of sample. Weigh 200 grams into a Waring Blender jar. Add 400 ml. of acetone and blend at high speed for 3 minutes. Make to 600 ml. with acetone and blend for an additional 2 minutes. Filter through a 32-cm., Whatman No. 12 fluted filter paper, collecting exactly 300 ml. of extract. Transfer to a 1-liter separatory funnel and extract with 300 ml. of Skellysolve B. Discard the lower aqueous phase and drain the Skellysolve B through a 250-gram bed of anhydrous sodium sulfate into a 600-ml. beaker. Rinse the sodium sulfate with an additional 50 ml. of Skellysolve B. Evaporate the solvent on a steam bath under an air jet to a volume of 100 ml. Filter the sample through a 32-cm. Whatman No. 12 fluted filter paper. Rinse the paper with two 25-ml. portions of Skellysolve B. Evaporate the combined filtrates just to dryness on a steam bath under an air jet.

Chromatography. Pour 100 ml. of a benzene slurry containing 30 grams of acid-washed alumina into a chromatographic column. Rinse down the sides of the column with small portions of benzene. When the alumina has settled, place a pledget of glass wool on top of the alumina. Dissolve the sample in 15 ml. of benzene and pour the concentrated extract into the column. Rinse the beaker with 15 ml. of benzene and apply this just as the last of the concentrated extract passes into the column. Repeat with a second 10-ml. benzene rinse. Finally, elute with an additional 75 ml. of benzene. Evaporate the effluent to 25 ml. on a steam bath under an air jet. Shut off steam and continue evaporation until the sample goes just to dryness.

Hydrolysis and Color Development. Take up the residue in 2 ml. of acetone. Transfer immediately to a 60-ml. separatory funnel. Complete the transfer with 7 ml. of concentrated ammonium hydroxide. Let the separatory funnel stand, with occasional shaking, for 10 minutes. Add 25 μl. of ammoniacal nickel solution. Let the sample stand, with occasional shaking, for 20 minutes, then add 8 ml. of acetone and 3 ml. of benzene to the funnel, and shake. If the aqueous phase remains cloudy, shake the funnel again. Repeat until the aqueous phase is clear. Drain the aqueous phase into a 10-ml. graduated cylinder. Tap the funnel to get out the small amount of liquid below the stopcock. Re-extract with 0.5 ml. of blank solution. Combine the wash and the original extract, and dilute to 10 ml. with blank solution. Determine the absorbance in an Evelyn colorimeter using a 540 mμ filter. When working with concentrated solutions of Morestan, the extraction of the nickel complex is not complete, as evidenced by the red color remaining in the benzene layer. In that case, re-extract the benzene with additional portions of blank solution until no further color can be extracted into the aqueous phase. Then dilute the combined extracts to volume with blank solution and apply the appropriate correction factor.

Preparation of Standard Curve. Pipet 0, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50 ml. of a 100 μg. per ml. stock solution into a series of 60-ml. separatory funnels. Dilute to 2 ml. with acetone. Add 7 ml. of concentrated ammonium hydroxide and proceed as described for the samples.

Discussion

Recovery Experiments. To test the efficiency of the over-all procedure, known amounts of Morestan were added to apples and pear samples which were then analyzed. The Morestan was added as an acetone solution at the initial blending step. The recoveries for apples at the 0.25 p.p.m. level were 91% and for pears at the 0.50 p.p.m. level, 84%.

Sensitivity. When the method described is used, an absorbance of 0.1 is obtained from 14 μg. (0.14 p.p.m.) of Morestan. This is considered to be the instrumental limit of sensitivity. However, inasmuch as control values were never greater than 0.05 p.p.m. (25 apple and 19 pear controls), the sensitivity could easily be increased to this level by using a Beckman DU spectrophotometer and 10-cm. (6-ml. volume) microcuvettes.

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