(8) Enkerlin-S., Dieter, Hanna, R. L., *Ibid.*, **49,** 560 (1956).
(9) Erickson, L. C., Brannaman, B. L., *Hilgardia* **23,** 175–84 (1954).
(10) Erwin, W. R., Schiller, Dora, Hoskins, W. M., J. AGR. FOOD CHEM. **3,** 676–9 (1955).
(11) Gunther, F. A., Blinn, R. C., Kolbezen, M. J., Barkeley, J. H., Harris, W. D., Simmon, H. S., *Anal. Chem.* **23,** 1836 (1951).
(12) Ivy, E. E., Brazzel, J. R., Scales, A. L., Martin, D. F., *J. Econ. Entomol.* **48,** 293–5 (1955).
(13) Jones, L. R., Riddick, J. A., *Anal. Chem.* **24,** 569–71 (1952).
(14) Kolbezen, M. J., Reynolds, T., J. AGR. FOOD CHEM. **4,** 522–5 (1956).
(15) Lloyd, E. P., Martin, D. F., *J. Econ. Entomol.* **49,** 764–6 (1956).
(16) Rainwater, C. F., *Agr. Chem.* **11** (2), 32–3, 107 (1956).
(17) Richmond, C. A., *Ibid.*, **49,** 874–5 (1956).
(18) Robertson, R. L., Arant, F. S., *J. Econ. Entomol.* **48,** 604–5 (1955).
(19) Wollenberg, O., *Angew. Chem.* **68,** 581 (1956).
(20) Wollenberg, O., Schrader, G., *Ibid.*, **68,** 41 (1956).

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INSECTICIDE RESIDUES

Colorimetric Determination of Heptachlor in Soils and Some Crops

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When heptachlor was determined in soils and some crops, extraction by tumbling, using a mixture of colorimetric pentane and acetone (4 to 1) and a subsequent cleanup with activated carbon, gave consistent results with recoveries of approximately 90%. The method is fast and allows a considerable saving of colorimetric pentane as compared with the method using a 10-gram Florex column for cleanup. Heptachlor in soils and crops was determined according to the method of Polen and Silverman.

HEPTACHLOR (1,4,5,6,7,8,8-heptachloro - 3a,4,7,7a - tetrahydro-4,7-methanoindene), after being isolated from soils or crops, was determined according to the Polen and Silverman method (*2*). It reacted with the Polen and Silverman reagent and the pink to violet color obtained was measured photometrically at 567 mμ against benzene–isopropyl alcohol (4 to 1) as a reference. Soils and various crops, treated with heptachlor in pentane, were extracted initially with colorimetric pentane in a Soxhlet extractor and passed subsequently through a 10-gram Florex column. Extremely low and erratic recoveries were encountered and reproducible results could not be obtained.

The principal loss of the heptachlor was in the chromatographic procedure. Batches of Florex obtained at different times varied considerably in their retention of both the heptachlor and interfering substances. Recoveries of known amounts of heptachlor added to Florex columns varied between 26 and 92%, with most recoveries being below 70%. When a mixture of colorimetric pentane–acetone in a 4 to 1 ratio was used, the recoveries were 11 to 15% higher than with an extraction with colorimetric pentane only. Crops or soils extracted with pentane and acetone gave an apparent heptachlor content of less than 0.1 p.p.m. Difficulties were also encountered with Soxhlet extractors, especially when soils had to be extracted. Inconsistent and sometimes low recoveries were obtained, most probably due to channeling.

The following procedure proved to be satisfactory.

Extraction Procedure

All materials under investigation were extracted by tumbling with a mixture of colorimetric pentane–acetone (4 to 1). Two-quart, wide-mouthed Mason jars were used and a 1-hour head to end tumbling was applied.

Soils. The amount of soil to be extracted depended on the amount of toxicant present. Where low residues were expected, more soil was taken. A maximum of 400 grams of soil could be extracted safely. The soil under investigation was extracted under moisture conditions similar to those prevailing in nature. With dry soil or soils which were too wet, extraction of the toxicant was incomplete. A mixture of colorimetric pentane–acetone (4 to 1) and anhydrous sodium sulfate, equal in weight to that of the soil, were added to the extraction jar. Two milliliters of solvent were used per gram of wet soil. An additional 100 grams of soil were dried for 24 hours at 50° C. to determine the dry weight of the soil.

Crops. The edible portion of the crop material was finely ground in a food chopper. Usually 100 grams were weighed out, placed on paper, and mixed with 200 grams of anhydrous sodium sulfate. During the drying time (about 0.5 hour) the crop and the sodium sulfate were mixed several times. When 100 grams of plant material were used for extraction, a mixture of 400 ml. of colorimetric pentane–acetone (4 to 1) was added to the crop–sodium sulfate mixture in the Mason jar. After tumbling, the extraction jars were placed into a refrigerator for 0.5 hour, to minimize the evaporation of pentane during filtration. After cooling, the supernatant liquid was decanted through glass wool. The recovered volume was recorded at room temperature to be used as a factor in the calculation of the results.

The acetone was removed from both soil and crop extracts by washing first with water and then with a saturated solution of sodium chloride. The extract was dried over sodium sulfate and concentrated to about 30 ml. on a 50° C. bath, using a Vigreux column.

Removal of Interfering Substances

From Soil Extracts, Including Muck Soil. Nuchar activated carbon (C 190–N, pH 6), 0.5 gram, was added to the concentrated extract. The mixture was swirled gently for 1 minute. After an additional 5 minutes, it was filtered through a $^1/_2$-inch layer of asbestos with glass wool on top. Chromatographic columns, 7 × $^3/_4$ inch with sintered glass disk bottoms, were used.

Slight pressure was applied when the filtration was slow. After several washings with colorimetric pentane from a wash bottle, the clear eluate was concentrated to approximately 15 ml. and then adjusted to 25 ml. in a volumetric flask. Aliquots of the whole extract, depending on the estimated amount of heptachlor present, were then used for analysis. This cleanup procedure allows a considerable saving of colorimetric pentane as compared with the method using a 10-gram Florex or Florisil column. As specific recovery tests were run with each analysis, the Nuchar used was tested each time.

From Plant Extracts. The plant material investigated consisted of beets, potatoes, carrots, and alfalfa.

Beet and potato extracts could be cleaned up according to the procedure used for soils. Carrots and especially alfalfa extracts, after being decolorized with 1 gram of Nuchar, had to be passed through a 4-gram Florisil column, to remove all interfering substances.

When a column of 10 grams of Florisil was used, the interfering substances were removed too, even without having added activated carbon to the extract.

With alfalfa, it seems to be advisable to remove waxes as described by Ordas, Smith, and Meyer (*1*).

Recoveries. Between 95 and 98% of known amounts of heptachlor, added to soils in a pentane or acetone solution, were recovered from a Miami silt loam and 85 to 92% from a muck soil. For crops recoveries between 87 and 93% were obtained.

Soils were usually treated on a 10-p.p.m. basis and crops on a 2-p.p.m. basis. The solvent in which the heptachlor was added to the soils or crops was evaporated before final extraction. The percentage figures for recoveries presented above are based on 23 recovery tests using a Miami silt loam, 9 tests with a muck soil, and between 8 and 17 recovery tests conducted with each of the four crops investigated.

Analytical Setup and Calculations

Each analysis was run in duplicate, using a soil or crop blank for determination of apparent heptachlor. In addition, known amounts of heptachlor were added to heptachlor-free soil or crop samples. The unknowns, after the value for apparent heptachlor had been subtracted, were calculated on the basis of the value obtained for the known amounts. As the effectiveness of the color reagent changes with time, analyze known amounts of heptachlor with each analysis. Results were expressed in parts per million, based on dry weight for soils and fresh weight for plants.

Literature Cited

(1) Ordas, E. P., Smith, V. C., Meyer, C. F., J. AGR. FOOD. CHEM. **4,** 444 (1956).

(2) Polen, P. B., Silverman, Paul, *Anal. Chem.* **24,** 733 (1952).

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FORAGE CONSTITUENTS

Detection of Saponins and Sapogenins on Paper Chromatograms by Liebermann-Burchard Reagent

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Developed and dried paper chromatograms of saponins or sapogenins are drawn through a warm bath of sulfuric acid and acetic anhydride (1 to 1, by volume) and spread on stainless steel. Characteristic colors usually begin to appear immediately and reach optimum intensity in about 15 minutes. Within this time very little background color develops. Colored photographic slides provide accurate records of the chromatograms.

OCCURRENCE OF triterpenoid saponins in alfalfa, ladino clover, and bur clover has been reported in earlier publications from this laboratory (*5, 9, 10, 11*). In further work with the same plants and other legume forages, need arose for a means to locate saponins and sapogenins on paper chromatograms.

The Liebermann-Burchard reagent (*4, 7*), a mixture of sulfuric acid and acetic anhydride, recommended itself, because it gives strong colors with many steroids and triterpenoids (*1, 3*). However, because of its destructive action, few attempts to use it on paper chromatograms appear to have been made (*2, 6, 8*). Trials of published methods failed to give satisfactory results with legume saponins and sapogenins and after some experimentation the procedure described here was devised.

Apparatus

35-mm. camera with Kodak Ektachrome F film and Wratten-82A filter.

Horizontal easel with adjustable light and camera supports (Figure 1).

Photoflood lamp bulbs No. RFL2.

Stainless steel sheet, 14-gage, one surface etched to dull finish with aqua regia. Suggested size 30 × 55 cm.

Procedure

The staining reagent is prepared by pouring acetic anhydride (ACS) into an equal volume of sulfuric acid (specific gravity 1.84). Mixing in the reverse order results in very rapid heating accompanied by violent sputtering and excessive darkening. Promptly after mixing, the reagent is cooled to about 80° C. by swirling the container in a stream of tap water. During mixing and cooling a small quantity of gas is evolved and the liquid becomes straw colored. The reagent is poured into an open container such as a glass evaporating dish, where it is allowed to cool to 70° C., at which temperature dipping may be started. The reagent deteriorates with use and a solution that has cooled below working temperature tends to become excessively dark and viscous if rewarmed. Thus, only quantities sufficient for immediate use should be prepared.

Oven dried chromatographic strips are drawn through the reagent at a rate of about 3 cm. per second and then for full color development they are laid on a sheet of stainless steel which has been warmed to about 40° C. A thermometer is used to submerge the papers as they pass through the bath and to check the temperature of the liquid, which should be between 60° and 70° C.

Stained areas corresponding to the locations of saponins or sapogenins begin to appear as red spots almost immediately. Stain intensification, which is accompanied by color changes to blue or purple with some substances, continues for about 20 minutes. The paper gradually disintegrates and darkens. However, if it is of a clean and firm textured grade such as Whatman's No. 3MM, its appearance changes but little within the time needed for optimum development of stains. Chromatograms of legume forage saponins and sapogenins are photographed 15 to 17 minutes after dipping.

The temperatures and times indicated are satisfactory for Whatman's 3MM paper. Softer papers need temperatures as low as 50° to 60° C. for the reagent, 25° C. for the plate, and periods up to 30 minutes for color development.

During stain intensification the reagent on the paper absorbs moisture from the air. The dilution that occurs in this way retards darkening of the papers by the action of concentrated sulfuric acid and is thus an essential feature of the staining process. Satisfactory re-