FUNGICIDE LABELING

Preparation of Labeled 2,6-Dichloro-4-nitroaniline (Botran)

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A procedure is described for the preparation of chlorine-36–labeled 2,6-dichloro-4-nitroaniline, Botran, by the chlorination of *p*-nitroaniline with the labeled chlorine gas.

THE compound 2,6-dichloro-4-nitroaniline (Botran, The Upjohn Co.) has been used on several fruits and vegetables for the control of certain fungi on a no-residue basis, and the use of tracer methods should facilitate the investigations of its residues in soils and plants. A carbon-14 label would be satisfactory, but the synthesis requires several steps from expensive labeled intermediates. The chlorine-36–labeled compound was found to be easily prepared, although the maximum specific activity obtainable is low.

p-Nitroaniline can be readily chlorinated with elemental chlorine-36 to form Botran:

$$NO_2C_6H_4NH_2 + 2\ Cl_2^* \rightarrow NO_2C_6H_2NH_2Cl_2^* + 2\ HCl^*$$

Experimental

The chlorine-36 gas in break-seal tubes, obtained from The Radiochemical Centre, England, had a specific activity of 44.2 microcuries per millimole. The apparatus for the synthesis is shown in Figure 1.

A mixture of 86 mg. (0.62 mmole) of *p*-nitroaniline and 2.5 ml. of glacial acetic acid was placed in the reaction tube, *B*, and the mixture warmed until the *p*-nitroaniline was dissolved. The break-seal tube containing 54 microcuries (1.22 mmoles) of chlorine-36 was joined to the connecting tube, *C*, and tube *B* was attached by means of the 24/40 joint.

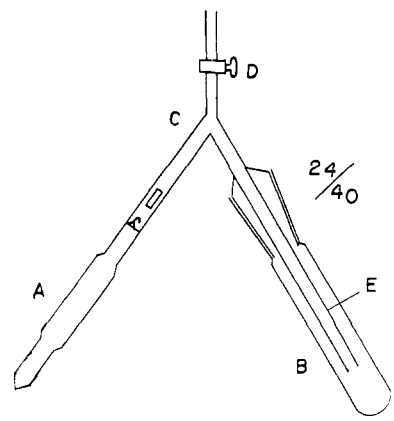

Figure 1. Chlorination apparatus

The lower parts of the break-seal tube and the reaction tube were immersed in liquid nitrogen. After the chlorine had condensed, the seal was broken, and the system was evacuated through *D*. The stopcock was closed and liquid nitrogen bath was removed from the break-seal tube and, as it warmed, the chlorine passed into tube *B* and was condensed. After the transfer was completed, air was admitted through stopcock *D*, tube *C* was disconnected from the reaction tube, *B*, and the latter was closed with a glass stopper. Tube *B* was then removed from the liquid nitrogen bath and allowed to warm to room temperature. The contents were shaken occasionally, and after the tube had stood an hour ice water was added until the tube was three fourths full, and the mixture was shaken. A milliliter of water dissolves about 0.8 mg. of *p*-nitroaniline, but only about 8 μg. of Botran. The mixture was filtered, and the product was washed with cold water. Half the chlorine used for the synthesis was in the form of HCl in the filtrate, and was saved for future use. The yield was about 80%, based on the weight of the Botran and the theoretical yield from *p*-nitroaniline. The product was recrystallized from a mixture of glacial acetic acid and alcohol (*1*, *2*). It melted at 193–195° C. alone, and mixed with Eastman Kodak No. 1033 2,6-dichloro-4-nitroaniline.

The labeled Botran had an activity of 440 counts per minute per microgram when counted in toluene containing 4 grams of PPO and 0.1 gram of POPOP per liter in a Model 3314 TriCarb scintillation spectrometer. No chlorine standard was available for efficiency determinations, but the counting efficiency was probably above 80%. Gas chromatography yielded only one peak, identical in elution time with the Eastman Kodak sample.

Literature Cited

(1) Witt, O. N., *Ber.* **8**, 143 (1875).
(2) Witt, O. N., Toeche-Mittler, S., *Ibid.*, **36**, 4390 (1903).

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FUNGICIDE RESIDUES

Determination of Lanstan Residues on Crops

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LANSTAN (1-chloro-2-nitropropane) is a broad-spectrum soil fungicide. It is particularly effective for the control of "damping off" diseases attacking emerging and emergent seedling cotton, *Fusarium* root rot of large-seeded legumes, and *Pythium ultimum* attacking sugar beets and red beets. The mode and rate of dissipation of Lanstan in soil are directed by the type of soil, temperature, amount of moisture, concentration, and other factors.

A sensitive method of analysis is required for registration of the compound for use on various crops.

The development of the method was complicated by the high volatility of Lanstan. The solution could not be concentrated by evaporation without substantial loss of the Lanstan.

Because of its extreme sensitivity and high selectivity, electron capture gas chromatography was found to be the best method for determining Lanstan in

A rapid gas chromatographic method was developed for determining Lanstan (1-chloro-2-nitropropane) residues on agricultural crops by electron capture detection. Minimal temperature conditions eliminate the necessity for an elaborate cleanup procedure. The general procedure is presented, with recovery and residue data from various crops.

crop extracts. This method is similar to the residue analyses of nematocides (*7*), which have properties similar to those of Lanstan.

Apparatus

A Jarrell-Ash 26–700 Universal chromatograph equipped with the 26-755 electron capture detector was used without modification. The column was a 4-foot, $^1/_4$-inch aluminum tube packed with 4% XE-60 on Chromosorb W (80-mesh). Prepurified nitrogen, dried by passing through 3 feet of Molecular Sieve 2A, was the carrier gas. The column was preconditioned at 190° C. for 3 hours and then held at 95° C. for 3 days before use.

Reagents

Lanstan Stock Solution. Dissolve 0.1 gram of Lanstan in 100 ml. of benzene.

Lanstan Working Standard Solution. Dilute 0.10 ml. of the stock solution to 100 ml. with benzene.

Florisil, 60- to 100-mesh. Use as received.

Check all reagents by the electron capture gas chromatograph for impurities before they are used.

Experimental

The detector was held at a constant temperature of 200° C. for optimum response with maximum life of the tritium source. The column was operated at 80° C. and the injection port at 120° C. The amplifier was set at 1×10^{-9} ampere sensitivity.

The maximum detector response for Lanstan was determined as shown in Figure 1. The maximum sensitivity occurs at a standing current of 4.5×10^{-9} ampere. The relationship between maximum sensitivity and standing current is constant, although the voltage may vary between successive days. The noise level at maximum sensitivity was 4×10^{-12} ampere.

Procedure

A 50-gram representative sample of crop is macerated in a homogenizer for 1 minute. The recovery samples are fortified before maceration with 1 to 5 μg. of Lanstan (1.0 to 5.0 ml. of working standard). One hundred milliliters of benzene and 50 ml. of methanol are added and the mixture is blended for 1 minute. The benzene layer is then separated, by centrifuging if necessary, washed with 100 ml. of water, and dried over sodium sulfate.

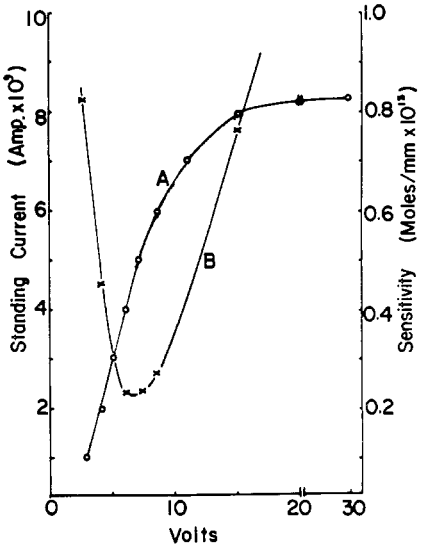

Figure 1. Detector characteristics

A. Standing current vs. applied voltage
B. Sensitivity to Lanstan (moles peak height) vs. applied voltage

An interference which occurs in some of the green vegetables (corn fodder, Brussels sprouts, sugar beet tops) can be removed by shaking the extract with Florisil (3 ml. per gram). The benzene should be saturated with water before this cleanup to ensure maximum recoveries.

Inject a 5.0-μl. aliquot of the benzene extract (2500-μg. crop) directly into the chromatograph which has been set at the previously determined conditions. Determine the standard curve before any extracts are injected into the instrument and check frequently during the day. Any change in column bleed, oven temperature, or flow rate will affect the calibration.

Dilute 10 ml. of the working standard to 100 ml. with benzene. Pipet 0-, 0.1-, 0.25-, 0.5-, 1.0-, 1.5-, and 2.0-ml. aliquots of this solution (0.1 μg. per ml.) into 10-ml. volumetric flasks and dilute to volume. Inject 5.0 μl. of each solution into the chromatograph. Plot peak height *vs.* picograms (pg.) injected to obtain the straight-line calibration curve.

Discussion

The injection port temperature was found to be a critical factor. Above 180° C., crop extractants were evidently pyrolyzed, giving a large amount of interfering fragments. Below 120° C., the peaks were broad and vague. The injection temperature was held to the minimum, which substantially reduced the amount of high boiling components leaving the injection port.

The injection port must be cleaned daily immediately after all crop extracts are analyzed. Heavy plant oils and waxes gradually bleed from the injection port and, after several hours, ruin the column. The aluminum injection block was easily cleaned by flushing with hot 10% nitric acid, followed by water, methanol, and hexane.

Under the operating conditions, Lanstan had a retention time of 3 minutes with excellent detector response. The column temperature was held at 80° C., which is 90° C. below the boiling point of Lanstan.

All chemicals must be checked daily for interferences. Sodium sulfate frequently contains traces of organic impurities and readily absorbs more from the atmosphere. Benzene must be distilled before use to remove high boiling impurities. All samples should be sealed tightly and kept from the chemical vapors usually occurring in the laboratory. Traces of water in the sample will change the detector characteristics and give anomalous results.

Dilute solutions are not very stable because of adsorption and chemical reactions which are usually insignificant in more concentrated solutions. For this reason, a "working" standard solution must be prepared daily from a more concentrated "stock" solution. The sample extracts, if not analyzed immediately, should be frozen until analysis time.

A method for the determination of Lanstan residues was developed using the Dohrman Microcoulometric gas chromatograph. Samples were extracted with benzene-methanol mixed solvent and the methanol was removed by washing with water. The benzene layer was evaporated to $^1/_{20}$ its original volume via air stream and dried. Two hundred microliters were injected into the instrument set at approximately the same conditions as the electron capture gas chromatograph. Recoveries ranged from 60 to 80%.

This method was inferior to the electron capture method because lack of sensitivity requires cleanup procedures, concentration of samples, and longer times of analysis. Difficulty was also encountered in venting the solvent, which eluted just prior to Lanstan. The volatility of Lanstan was apparent, since