Electron Affinity Residue Determination of CIPC, Monuron, Diuron, and Linuron by Direct Hydrolysis and Bromination

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An analytical method is described for the determination of residues of CIPC, monuron, diuron, and linuron. After extraction from fruits and vegetable crops with acetone and partitioning into hexane, the herbicides are directly hydrolyzed and brominated to yield their respective brominated anilines. These derivatives are determined by electron affinity gas chromatography. Recovery of the herbicides from fruit and vegetables is good. The method is sensitive to about 0.02 p.p.m. of the herbicides.

CIPC, monuron, diuron, and linuron are widely used as herbicides in fruits, vegetables, and other crops. Although these herbicides contain chlorine, they are not sufficiently sensitive for direct electron affinity residue determination without prior cleanup and concentration. This is, presumably, due to the presence of the chlorines on a benzene ring, the pi electrons of which stabilize the halogen.

Bromoorganic compounds have a much greater electron affinity than the corresponding chloro analogs. In the work reported, acetone extracts of crops are partitioned into hexane and directly hydrolyzed and brominated in one step. The resulting brominated anilines are then determined by electron affinity gas chromatography. Bromination of the chloroanilines is facilitated by the strong ortho, para-directing influence of the amino group for bromine substitution.

Equipment

The gas chromatograph used was a Barber-Colman Model 10 with a battery-operated (*2*, *3*), Barber-Colman No. A-4071, 6-cc. detector containing 56 μc. of radium-226. The detector was operated at 5 volts, which was found to be optimum for electron capture by halogenated compounds. A 90,000-megohm resistor was added to the electrometer to give additional gains of 3,000; 10,000; and 30,000. The 3,000 setting was used exclusively in this study. A 0–50 mv. Wheelco Recorder equipped with 10-inch chart paper running 10 inches per hour was used.

The column was borosilicate glass, U-shaped, 9-mm. o.d., and 6 feet long. The packing was 5% ethyl acetate-fractionated, Dow Corning high vacuum silicone grease on 80- to 100-mesh, acid-washed Chromosorb W. Connections between the column and detector were made with metal hypodermic tubing, glass elbows, and silicone rubber through-septums. The operating temperatures for the column, flash heater, and detector were 200°, 265°, and 235° C., respectively, and nitrogen (60 cc. per minute) was the carrier gas. The column was conditioned for 16 hours at 230° C. before use.

Procedure

CIPC [Isopropyl *N*-(3-Chlorophenyl) Carbamate] in Potatoes. Blend several potatoes in their own liquid until a well-mixed puree is obtained. Transfer 25 grams of the mixture to a semimicro Waring Blendor jar and add 70 ml. of distilled acetone. Blend the mixture for 2 minutes. Transfer the contents to a sintered-glass funnel (40-mm., coarse porosity disk) upon which a thin layer of glass wool has been placed. Filter the sample by suction into a 125-ml. flask marked at approximately 100 ml. Rinse the filter with two 20-ml. portions of acetone each time compressing the sample with the bottom of a 50-ml. beaker to squeeze out remaining acetone. Pour the filtrate into a graduated cylinder and adjust the volume to 100 ml. by evaporation with an air stream or addition of acetone.

Transfer 5 ml. of the acetone solution to a 100-ml. volumetric flask and add 10 ml. of distilled Skellysolve B. Make the flask to volume with 2% sodium sulfate and shake vigorously for 1 minute. Transfer 1 ml. of the upper hexane layer to a 10-ml. volumetric flask and evaporate the solution to dryness with a gentle air stream. Add the following solutions to the flask: 1 ml. of glacial acetic acid, 5 drops of 9*M* sulfuric acid, and 0.2 ml. of a solution of acetic acid saturated with iodine crystals and containing 5% (by volume) of liquid bromine. Mix the contents and add a carborundum boiling chip to the flask. Place a 10/30 standard taper male ground joint (with the full 14.5 cm. length of glass tubing attached) in the top (as an air condenser). Immerse the flask to a depth of 1 cm. in a 130° C. constant temperature oil bath for 1 hour.

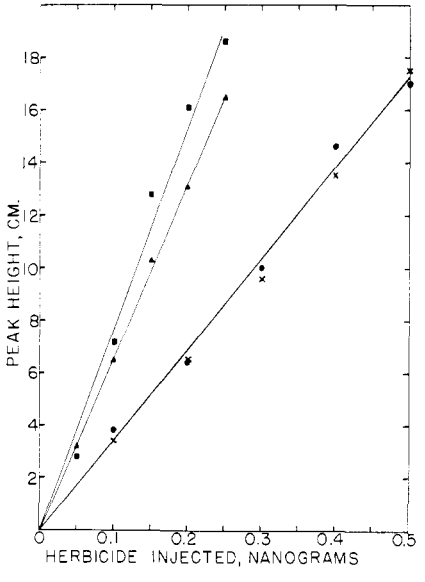

Figure 1. Standard curves of CIPC (▲——▲), monuron (■——■), diuron (●——●), and linuron (x——x)

Then remove the flask and rinse down the inside of the condenser and joint into the flask with about 4 ml. of distilled water. With the condenser removed, cool the flask further in a beaker of cold tap water while more adding 3 ml. of 50% sodium hydroxide with constant swirling. Immediately add 1 ml. of distilled hexane to the flask, make to volume with water, and shake the contents vigorously for 1 minute. Inject 10 μl. of the upper hexane layer into the column. The retention time for the resulting 2,4,6-tribromo-3-chloroaniline is approximately 11 minutes.

When several samples (or standards) are being analyzed, each flask must be placed in the oil bath at 10-minute in-

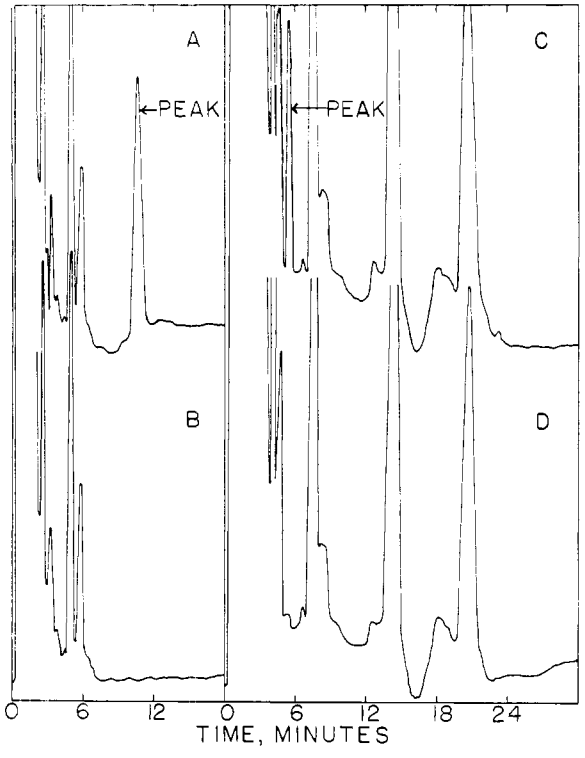

Figure 2. Chromatograms of (*A*) CIPC-treated potatoes, (*B*) untreated potatoes, (*C*) Cattawba grapes to which 0.2 p.p.m. of monuron was added, and (*D*) untreated grapes

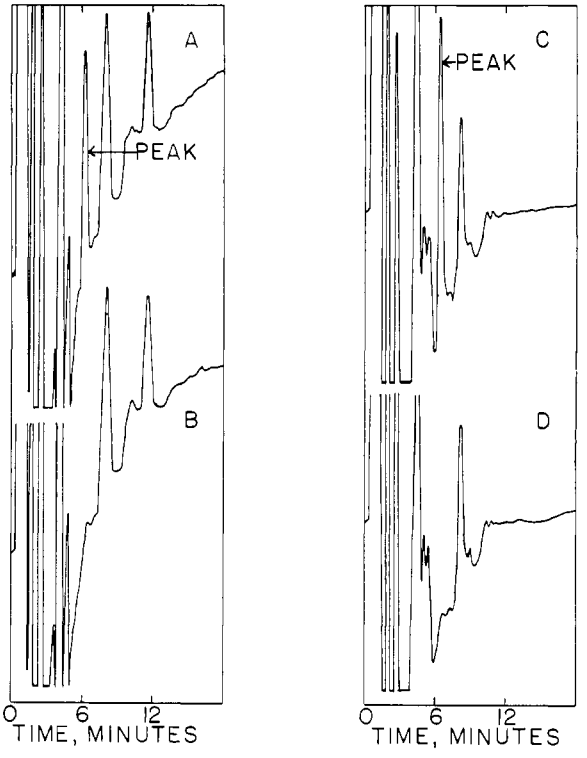

Figure 3. Chromatograms of (*A*) purple raspberries containing 0.1 p.p.m. of added diuron, (*B*) untreated raspberries, (*C*) carrots containing 0.1 p.p.m. of added linuron, and (*D*) untreated carrots

tervals. This will allow sufficient time for immediate cooling, neutralization, and extraction into hexane at the end of the hydrolysis. The resulting brominated aniline of CIPC is stable (up to 24 hours) after extraction into hexane. It apparently decomposes, however, if allowed to stand in the aqueous acid solution. This is also true for the brominated anilines of monuron, diuron, and linuron.

The standard curve for CIPC is developed as follows. Pipet 0, 0.1, 0.2, 0.3, 0.4, and 0.5 ml. of CIPC (0.05 μg. per ml.) in acetone into a series of 10-ml. volumetric flasks. Evaporate the solutions with air and proceed as in the analysis of potatoes beginning with the addition of 1 ml. of glacial acetic acid.

Monuron (3-*p*-Chlorophenyl-1,1-dimethylurea) in Grapes. The procedure for monuron is identical to that for CIPC with the following exceptions. The nitrogen flow rate through the column is 40 cc. per minute. After extraction of grapes with acetone, 5 ml. of the acetone solution are transferred to a 100-ml. volumetric flask and evaporated with air. Ten milliliters of benzene are added, the flask is made to volume with 10% sodium sulfate, and shaken for 1 minute. One milliliter of the upper benzene layer is then evaporated in a 10-ml. volumetric flask and carried on through the procedure. The retention time for the resulting 2,6-dibromo-4-chloroaniline is approximately 5.5 minutes.

Diuron [3-(3,4-Dichlorophenyl)-1,1-dimethylurea] in Raspberries and Linuron [3-(3,4-Dichlorophenyl)-1-methoxy-1-methylurea] in Carrots. The procedure for these herbicides is the same as for monuron with the following exceptions. The nitrogen flow rate is 60 cc. per minute. Five milliliters of benzene and a 50-ml. volumetric flask are used for the initial partition step. One-tenth milliliter of the bromine-iodine solution is used instead of 0.2 ml. The standard curve is developed using 0, 0.2, 0.4, 0.6, 0.8, and 1.0 ml. of either herbicide (0.05 μg. per ml.) in acetone. The retention time for the 2,6-dibromo-3,4-dichloroaniline (the brominated hydrolysis product of either herbicide) is approximately 6 minutes.

Results and Discussion

Figure 1 shows typical standard curves for the herbicides. A single line has been drawn to represent both diuron and linuron. These herbicides differ in molecular weight by only 16 units, and since hydrolysis of either yields 3,4-dichloroaniline, the curve for diuron would be only slightly steeper.

In Figure 2, chromatograms are shown of CIPC-treated potatoes (*A*), (the peak is at 11 minutes, representing 2.6 p.p.m. of the herbicide), untreated potatoes (*B*), Cattawba grapes containing 0.2

Table I. Recovery of Herbicides Added to Fruits and Vegetables

Amount Added, P.P.M.	Recovery, %
CIPC in Potatoes	
0.05	76, 92
0.1	95, 80
0.6	84
1.2	89
Monuron in Cattawba Grapes	
0.06	80, 107
0.1	79, 104
0.2	84, 92
0.4	81, 87
Diuron in Purple Raspberries	
0.1	92, 118
0.2	77, 81, 105
0.5	96, 116
Linuron in Carrots	
0.1	90, 116
0.2	92, 98
0.5	107, 112

Table II. Comparative Residue Values for CIPC in Bin-Treated Potatoes

Sample	Residue, P.P.M. Electron affinity	Residue, P.P.M. Colorimetric
Medium II	4.6	5.6
Medium VI	3.5, 3.5	4.1
Medium VII	3.4	3.8
Medium VIII	2.6	2.5

p.p.m. of added monuron (*C*) (the peak is at about 5.5 minutes), and untreated grapes (*D*).

The potatoes were treated in their storage bins to control sprouting. Figure 3 shows chromatograms of purple raspberries with 0.1 p.p.m. of diuron added (*A*), untreated raspberries (*B*), carrots with 0.1 p.p.m. of linuron added (*C*), and untreated carrots (*D*). The peak in the case of each herbicide appears at approximately 6 minutes.

The recoveries of the herbicides are listed in Table I. Acetone solutions of the herbicides were added to the crops in the Waring Blendor. For the recovery of the herbicides added above the 0.1 p.p.m. level, the acetone strip solutions were appropriately diluted before partitioning into hexane or benzene. The method is sensitive to about 0.02 p.p.m. of any of the herbicides. This concentration would yield peak heights equal to at least a 5% full-scale deflection with a 10-μl. injection. Table II lists the comparative residue values obtained by electron affinity and colorimetric (*1*) analysis of potatoes treated in bins with CIPC to control sprouting. Analysis of diuron in raspberries which received a foliar-soil application of 3 and 6 pounds per acre of the active herbicide (as an 80% wettable powder) in April and May and were harvested in July, 1962, showed no detectable residues.

All glassware used in the procedure was cleaned in dichromate–sulfuric acid solution and thoroughly rinsed. This is necessary to remove any oxidizable organic contaminents which can cause reduction of bromine and iodine. Stock and standard solutions of the herbicides must be stored in clean glassware. Alkaline or acid contaminants can frequently initiate hydrolysis to the anilines. The solutions may then turn yellow or reddish brown owing to oxidation and polymerization reactions leading to quinone-type compounds.

A constant temperature of 130° C. for 1 hour during hydrolysis and bromination is essential. Each flask must then be removed and immediately cooled, neutralized, and partitioned into hexane.

At constant flow rate and temperature, the brominated anilines of the above herbicides show the following order of increasing retention time: monuron < diuron = linuron < CIPC. This order would be expected from the molecular weights of the brominated anilines assuming ortho and para bromination. In strongly acid solution, the NH_3^{+1} ion can exist which is very weakly meta-directing for bromination. The *m*-bromoanilines of these herbicides would, however, (based on molecular weight) indicate the following order of increasing retention times: CIPC < diuron = linuron < monuron. This is the exact reverse of the experimentally determined retention times. Apparently, the initial presence of the electrophilic chlorine substituents on the aniline ring sufficiently reduce the basic strength of the amino group to prevent formation of the NH_3^{+1} group in the acetic-sulfuric acid hydrolysis solution. The amino group would therefore exist to promote ortho and para bromination.

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INSECTICIDE METABOLISM

The Metabolism of Dimethoate by Vertebrate Tissues

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THE INSECTICIDE DIMETHOATE [*O,O*-dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorodithioate] is of interest as a compound of low mammalian toxicity with systemic activity in animals and plants. It is metabolized initially in lactating cows and male rats (*3*) and in sheep (*2*) to give primarily dimethoate acid (*O,O*-dimethyl *S*-carboxymethyl phosphorodithioate), somewhat less *O,O*-dimethyl phosphorodithioate, and various other products (*3*). Dimethoate is broken down much more rapidly in the mouse than in the housefly or American cockroach, and this difference has been suggested to be the cause of its selectivity (*7*). It is 70 times more toxic to the cockroach and 325 times more toxic to houseflies than it is to mice (*7*).

The term "selectophore" was introduced (*9*) in 1960 to apply to a chemical group whose presence in a molecule conferred selectivity, usually by permitting an enzyme which is particularly effective in one class of animals to degrade the poison to ineffective products. The validity of such a concept has been amply shown for the case of the carboethoxy group in malathion [*O,O*-dimethyl *S*-(1,2-dicarboethoxyethyl) phosphorodithioate], which is hydrolyzed more rapidly in mammals than in insects at a carboethoxy group to yield the corresponding carboxyacid, which is a poor anticholinesterase because of its anionic character. Is the amide group of dimethoate a selectophore? Evidence in favor of the hypothesis is that EPN (ethyl *p*-nitrophenyl phenylphosphorothionate) and TOCP (tri-*o*-cresyl phosphate) synergized the toxicity of dimethoate to mice (*10*), and this has been shown to constitute good evidence that carboxyesterase or carboxyamidase is responsible for low toxicity (*10*).

Let us define "selectophore enzyme" as the enzyme which operates upon the selectophore. It is the purpose of this paper to determine whether an amidase is the selectophore enzyme for dimethoate, and to establish its location

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