Pentachloronitrobenzene Residues in Potatoes

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Gas-liquid chromatography has been applied to the analysis of pentachloronitrobenzene (PCNB) in potatoes grown in PCNB-treated soil. PCNB residues are mainly in the peel. One of two metabolites detected was shown to be pentachloroaniline (I). The second remained unidentified. Pentachloroaniline was formed during fermentation from PCNB added to potato homogenates.

Until a few years ago the determination of pentachloronitrobenzene (PCNB) residues in potatoes, after its application against *Rhizoctonia solani* for instance, was carried out almost exclusively by photometry (Ackermann *et al.*, 1958; Ackermann *et al.*, 1963; Klein and Gajan, 1961) or polarography (Klein and Gajan, 1961; Webster and Dawson, 1952; Bache and Lisk, 1960; Gorbach, 1961).

Both methods are specific for the nitro group. Derivatives of PCNB, such as may be produced in the plant's metabolism, do not therefore register with these methods if the nitro group is not present in the derivative.

The probability of detecting such compounds is the greater when one makes use of gas chromatography. Metabolites that have a similar or lower boiling point than PCNB may be registered by this method. The gas chromatographic determination of PCNB is already well documented (Klein and Gajan, 1961; Burke and Holswade, 1964; Cassil, 1962).

In this paper, the authors report results obtained in the investigation of residues in potatoes grown in PCNB-treated soil in the field. The active residues were extracted with a mixture of 2-propanol and benzene, and the determination was carried out by gas chromatography in extracts washed with sodium chloride solution. The detector used was the halogen specific coulometric detector (Klein and Gajan, 1961; Cassil, 1962; Coulson *et al.*, 1960).

Results

The PCNB residues (Table I) were mostly in the peel and in the 1- to 2-mm. thick cellular tissue underneath the peel. The interior of the potato, on the other hand, was practically free from PCNB residues.

In addition to the peaks assigned to PCNB, the gas chromatograms exhibited two others, which had the relative retention volumes $V_{ik}^{\circ} = 1.3$ and $V_{ik}^{\circ} = 0.9$ (PCNB, $V_{ik}^{\circ} = 1$) (hereinafter referred to as metabolites I and II), and which were not present in the chromatograms of the untreated controls.

The chlorine-specific coulometric detector permitted the estimation of the quantity of the two unknown components in the chromatograms from their chlorine content (Table I under metabolites I and II).

Even in potato homogenates, to which PCNB had been added, a metabolite could still be found, identifiable by the retention time $V_{ik}^{\circ} = 1.3$, when the homogenate had fermented. Under sterile conditions no decomposition was noticed. About 8 mg. of metabolite I were isolated from such a fermentation batch (500 grams) by thin-layer chromatography and identified as pentachloroaniline by infrared and mass spectra. The pentachloroaniline was relatively nontoxic and had an LD_{50} (rat) of 15 grams per kg. (Weigand, 1966). The PCNB was largely (about 95%) decomposed under fermentation conditions and at the conclusion of the tests (lasting 50 to 80 days on the average) was present as 80% of pentachloroaniline in the mean.

Metabolite II, characterized by the retention time $V_{ik}^{\circ} = 0.9$, did not increase in concentration, in contrast to the pentachloroaniline, and as a result was only present in small, roughly constant concentrations (pentachloroaniline to metabolite II, 1000 to 5) and could not as yet be isolated or identified. It is probably an intermediate in the reduction of PCNB to pentachloroaniline. In contrast to the findings with the homogenates, the potatoes in the field experiments contained a roughly similar or larger quantity of metabolite II than metabolite I (pentachloroaniline).

On the other hand, after 170 days, humus soil, which was fortified with 50 p.p.m. of PCNB, showed that the main metabolite was the one with the relative retention time $V_{ik}^{\circ} = 1.3$. Another one had the retention time $V_{ik}^{\circ} = 1.8$ and is still unidentified. The $V_{ik}^{\circ} = 0.9$ metabolite was not detected. One may conclude from these results that the metabolite is formed before penetrating the potato's peel.

Pentachloroaniline behaves in exactly the same way in both gas and thin-layer chromatography (Table II), as does metabolite I in the potato skins. The color reaction of metabolite I with naphthyl-ethylenediamine dihydrochloride on thin-layer chromatographic plates after reaction with nitrous gases also points to the presence of the amino group, so that metabolite I may be considered as identical with pentachloroaniline. Metabolite II showed no color reaction under these conditions and accordingly contains no free amino group.

Method

Determination of Pentachloronitrobenzene Residues in Potatoes. The potatoes are prepared by washing and peeling for investigation and cut to shreds about 0.5 cc. in size. One hundred grams of sample are then mixed in a beaker with about 100 grams of anhydrous

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Table I. Residues of PCNB and Metabolites I and II in Potato Peels and Inner Parts

Quantity Applied, Kg./Hectare	Peel, μg./G.			Inner Parts,[a] μg /G	
		Metabolite		Metabolite	
	PCNB	I	II	I	II
25	0.20	0.04	0.04	<0.01	<0.01
	0.20	0.04	0.04	nd[b]	<0.01
50	0.40	0.06	0.09	<0.01	0.02
	0.24	0.1	0.1	nd[b]	0.01
100	1.4	0.3	0.2	<0.01	0.04
	1.0	>5.!	0.2	0.01	0.03
200	1.2	0.1	0.2	0.02	0.06
	1.7	0.2	0.3	...	0.06
400	3.0	0.1	0.2	<0.01	0.06
	3.0	0.3	0.2	<0.01	0.07
800	3.0	0.2	0.2	0.01	0.07
	4.0	0.4	0.2	<0.01	0.1

[a] PCNB was not detectable in any of the inner parts.
[b] Not detectable. For the peels the median value of the blank, the untreated sample was: PCNB, 0.1 ± 0.03 μg./g., metabolites I and II, 0.04 ± 0.02 μg. Cl/g.; for the interiors: PCNB and metabolites I and II, 0.01 ± 0.005 μg. (PCNB or Cl)/g.
Brassicol, superconcentrated (60% pentachloronitrobenzene) was used. Harrowing 4 days before sowing; harvesting 5 months after sowing. Storage of potatoes until analysis: 1 month at +4° C.; Variety: Binjte; Soil: Sandy loam; Country: Denmark; Cultivation by Statens plantepatolokiske forsøg, Lyngby.

sodium sulfate, and the mixture is treated with 150 ml. of benzene and 2-propanol (2 to 1). The resulting mixture is then homogenized in a mixer and allowed to settle, and the supernatant solution is filtered into a separatory funnel through glass wool. The sample is extracted twice more with 75-ml. portions of the extraction solvent, and the resulting mixture is filtered. Finally the combined extracts are transferred to the separatory funnel.

Two hundred milliliters of 2% sodium chloride are added to the solution in the separatory funnel, and the funnel is shaken for about 2 minutes—not too violently. After the phases have separated, the lower aqueous phase is run off and discarded. About 4 to 7 grams of anhydrous sodium sulfate are added to the remaining benzene phase, the funnel is rocked gently until the benzene phase becomes clear—adding further sodium sulfate if necessary—and the benzene is filtered into an evaporating dish through a paper filter. The filter is rinsed with a little benzene (dripped onto the filter paper edge). The benzene phase is then concentrated to 8 ml. under an infrared lamp and with ventilation. If there is any great risk of contamination from the air in the laboratory, a closed rotary evaporator is to be preferred. The concentrated solution is made up to a final volume of 10 ml. The subsequent determination by gas chromatography shows whether the solution should be further diluted or concentrated.

Gas Chromatographic Determination. The equipment consists of a Dohrmann Coulometer C 200 and the chlorine cell T 300 with suitable gas chromatograph. The latter is a self-assembled piece of equipment, whose special feature is the mounting of the injection block, the column, and the combustion chamber on the inside of the door of a commercial thermostat from Heraeus (Hanau), Type FTU 340. The combustion chamber is thus included in the thermostatically controlled system. The system—injection, column, combustion tube outlet—consists entirely of quartz. The sample is injected directly onto the column. The quartz column is so arranged that it extends through the injection port onto the septum. The gas inlet pipes are of flexible Ermeto tubes. Eluted components may be pyrolyzed or allowed to escape as required, via a T-piece inserted between the combustion chamber and the column and having a stopcock outside the thermostat.

Table II. R_f Values of PCNB and Metabolites I and II on Thin-Layer Chromatoplates

Solvent System	R_f[a], PCNB	R_f[a], Metabolite I	R_f[a], Metabolite II	Pentachloroaniline
Chloroform + benzene 1 to 1 (v./v.)	0.73	0.67	0.77	0.67
CCl_4	0.52	0.36	0.69	0.36

[a] The R_f values are for silica gel GF_{254} layers, 0.3 mm. at 25° C. in a saturated chamber.

Experimental

Column: 1-meter quartz, 4-mm. i.d. filled with silicone grease (according to Cassil, 1962) on Chromosorb W.

Temperature: 170° C.

Carrier gas: nitrogen at 1.6 atm.

Oxygen: 4.3 liters per hour.

Temperature of combustion chamber: 800° C.

Results were computed by the theoretical method described by Cassil (1962) which includes the recorder sensitivity, chart speed, and resistance of the coulometer output in ohms. The yield of the technique is 80 ± 10%, the limit of detection about 50 nanograms of PCNB; in practice, however, this is determined by the blank value and its standard deviation.

The result is stated as "nd" if the net value remaining

after subtraction of the blank value is less than half the blank value standard deviation. The result is "less than" when the value is less than the tripled blank value standard deviation, but greater than half of the blank value standard deviation. Net values, which are greater than the tripled blank value standard deviation, are stated as such.

Isolation of Metabolite I (Pentachloroaniline) from Potato Homogenates

Five hundred grams of unpeeled potatoes were homogenized in a mixer and 50 mg. of PCNB—dissolved in 2 to 3 ml. of ethyl alcohol—added to the homogenate. The mixture was allowed to stand at 25° C, and the fermentation starts about 24 hours later. After 5 to 10 days, sufficient pentachloroaniline (40 to 50% of the PCNB employed) has been formed. This quantity can be determined by gas chromatography as described in the determination of PCNB. To isolate the metabolite, one proceeds at first in accordance with the first method, but takes a 300-gram sample and, as a consequence, three times the stated quantities of reagents. The resulting benzene solution is concentrated as far as possible in a rotary evaporator, and the concentrate is applied to the start line of several thin-layer chromatographic plates. The plates are developed with carbon tetrachloride.

The zones between the R_f values 0.3 to 0.4 contain the pentachloroaniline. The zones show up as dark stripes on a greenish fluorescing background when silica gel GF_{254} is used and may thus be detected and scraped off in each chromatogram. The gel thus removed is extracted with benzene on a sintered glass filter and the extract evaporated in a rotary evaporator until the first signs of dryness. Further purification takes place via sublimation in high vacuum in the cold finger apparatus at 150° C. The cold finger is cooled with a mixture of dry ice and acetone

The infrared spectrum of the sublimate (KBr tablet) has the pentachloroaniline characteristic bands at 682, 763, 1090, 1123, 1265, 1375, 1418, 1592, 3380, and 3460 $cm.^{-1}$ (Perkin-Elmer, Model 21). The mass spectrum was identical with that of pentachloroaniline (AEI, M.S. 9).

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