Direct Analysis of Carbofuran and Two Nonconjugated Metabolites in Crops by High-Pressure Liquid Chromatography with UV Absorption Detection

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Carbofuran and the nonconjugated forms of 3-hydroxycarbofuran and 3-ketocarbofuran were extracted from several spiked foods by blending with acetone. The extract was partitioned into hexane–methylene chloride and passed through a 2% deactivated Florisil column for cleanup. The pesticide fractions were evaporated to near dryness and dissolved in 2,2,4-trimethylpentane (TMP, isooctane) for high-pressure liquid chromatographic (HPLC) analysis on a 25 cm × 2.2 mm (i.d.) LiChrosorb Si 60 (5 μm) column. Mobile phase composition varied between 3–8% (v/v) 2-propanol in TMP depending upon the compound and food analyzed. Detection was carried out at 254 nm for 3-ketocarbofuran while 280 nm was used for carbofuran and 3-hydroxycarbofuran. Detection limits in the foods studied were about 0.05 ppm for carbofuran and 3-hydroxycarbofuran and 0.02 ppm for 3-ketocarbofuran. Recoveries ranged from 68–110% for the foods and compounds studied at 0.1 and 1.0 ppm.

Carbofuran and two of its metabolites, 3-hydroxycarbofuran and 3-ketocarbofuran, have been determined by several methods based on gas chromatography. Direct analysis of carbofuran and 3-hydroxycarbofuran has been carried out in corn using microcoulometric detection (Cook et al., 1969). Williams and Brown (1973) applied this method to the analysis of the same two compounds in small fruits but using electrolytic conductivity detection. These nitrogen-selective detectors proved very useful for detecting residues as low as 0.025 ppm. However, we have found that the direct gas chromatography of carbofuran, and even more so 3-hydroxycarbofuran, is not easily accomplished. Columns must be well conditioned and GLC parameters have to be well controlled in order to obtain optimum response. Maintaining such specialized conditions for one or two particular pesticides is often not possible in pesticide laboratories where a large number of different pesticides must be analyzed under various GLC conditions. Thus, several methods of carbofuran analysis have arisen which make use of suitable derivatives for GLC. These products have two advantages over the direct GLC methods in that they are usually much more sensitive and that because they are chromatographically stable, a range of GLC parameters can be used. Butler and McDonough (1971) used hydrolysis followed by trichloroacetylation of the phenols for the determination of carbofuran, 3-hydroxycarbofuran, and 3-ketocarbofuran in a number food crops. Trifluoroacetylation (Wong and Fisher, 1975; Lau and Marxmiller, 1970; Seiber, 1972) of the intact carbamates has been studied for electron-capture detection. Heptafluorobutyryl derivatives of carbofuran and the two metabolites have also been evaluated by both electrolytic conductivity and electron-capture detection (Lawrence and Ryan, 1977; Lawrence et al., 1977; Seiber, 1972) and appear to be superior to the trifluoroacetyl derivatives in both terms of sensitivity and stability.

The disadvantages of derivatization methods are that they require extra time for derivative formation and increase the chance of error due to the extra sample manipulation. Also, for quantitative measurements the yield of product under the influence of the sample matrix must be the same as that obtained from a pure standard pesticide in order to make an accurate estimate of the pesticide concentration in the sample. Thus, spiked "blank" samples have to be frequently analyzed to ensure reproducible derivative formation.

We describe in the following paragraphs an attempt to analyze carbofuran and the nonconjugated forms of 3-hydroxycarbofuran and 3-ketocarbofuran directly by high-pressure liquid chromatography (HPLC) with ultraviolet absorption detection. The advantages over existing methods for these compounds is that, unlike direct GLC, all three are very stable, producing reproducible responses under a variety of HPLC conditions. Also, because it is direct, errors involved with derivatization are avoided as well as the additional time required for derivatization. Finally, all three compounds are detectable in low nanogram quantities making possible the application of this method to the detection of residues at levels as low as 0.1 ppm or less in several food crops.

EXPERIMENTAL SECTION

Apparatus. The major components of the HPLC system were a Waters Associates Model 6000A pump and a Waters Model 440 UV detector with dual cells (254 and 280 nm) which was connected to a dual-pen strip-chart recorder (1 mV). The chromatography column (25 cm × 2.2 mm i.d.) was packed in our laboratory with LiChrosorb Si 60 (5 μm) by a balanced density slurry technique (Cassidy et al., 1974). Samples were injected on the column via a modified Valco sample-loop injector which permitted syringe injection at ambient pressure (Cassidy, 1976). The mobile phase consisted of 2-propanol in 2,2,4-trimethylpentane (TMP) at concentrations of 3–8% depending upon compound and food analyzed. The flow rate was 1.0 mL/min for all analyses.

Reagents. All solvents were glass-distilled, residue-free materials. Stock solutions of carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate), 3-hydroxycarbofuran (2,3-dihydro-3-hydroxy-2,2-dimethyl-7-benzofuranyl methylcarbamate), and 3-ketocarbofuran (2,3-dihydro-2,2-dimethyl-3-oxo-7-benzofuranyl methylcarbamate) were prepared in 2-propanol (1 mg/mL). Working solutions were prepared by dilution with TMP. The crops studied were carrots, cabbage, corn, peas, potatoes, turnip, and wheat.

Sample Extraction. A 35-g representative sample of washed, chopped food was spiked with the carbamates and then blended with 100 mL of acetone for 4 min at medium speed with a Sorvall homogenizer. The mixture was filtered with suction through a 150-mL medium porosity

sintered glass funnel into a 500-mL suction flask. The filter cake was rinsed with about 10 mL of acetone. The filtrate was transferred to a 500-mL separatory funnel which contained 200 mL of a 1:1 (v/v) solution of hexane and methylene chloride. The contents were shaken for 2 min, then the aqueous (lower) phase was drawn off into a 250-mL separatory funnel containing 15 mL of saturated NaCl solution. This mixture was extracted twice with 70-mL volumes of methylene chloride. The organic extracts from the three partitions were combined and dried with 5 g of anhydrous Na_2SO_4 for at least 10 min. The dried extract was filtered through a 150-mL sintered glass funnel with suction into a 1-L round-bottom flask. The Na_2SO_4 was rinsed with 10 mL of methylene chloride. The combined extract was then evaporated by rotary evaporation at 30 °C to not less than 0.5 mL and transferred to a graduated centrifuge tube with several rinsings of 30% methylene chloride in hexane and adjusted to 7 mL. A 1-mL aliquot (5 g) was used for Florisil cleanup.

Florisil Cleanup. Two percent deactivated Florisil was prepared by adding doubly distilled water to Florisil which had been activated at 130 °C for at least 18 h and cooled to room temperature. The mixture was mechanically shaken for 8 h and permitted to stand in a well-sealed jar for 24 h before being used. A 5-g quantity of the deactivated Florisil was placed into a 1.5-cm i.d. glass buret containing a wad of glass wool in the bottom. About 1 g of Na_2SO_4 (anhydrous) was added to the top of the Florisil. The column was rinsed with 50 mL of hexane, followed by addition of the sample extract. The column was then eluted with 25 mL of 30% CH_2Cl_2 in hexane which was discarded. Carbofuran and 3-ketocarbofuran then were eluted with 55 mL of 15% acetone in hexane. After this, another 10 mL of 15% acetone in hexane solution was passed through the column and discarded. Finally the 3-hydroxycarbofuran was eluted with a further 70 mL of the same solvent mixture. Each fraction containing the carbamates was evaporated to a volume of about 0.5 mL (not to dryness since losses will occur) by rotary evaporation at 30 °C. The residue was quantitatively transferred to a 5-mL glass-stoppered graduated centrifuge tube with 3% 2-propanol in TMP. The sample then was diluted to an appropriate volume for HPLC analysis. Normally 20–25 µL was injected. The mobile phase most frequently used for carbofuran was 3% 2-propanol in TMP, although 5% was occasionally used when samples contained no interfering peaks. For 3-hydroxycarbofuran, 8% was found most useful. 3-Ketocarbofuran was analyzed either with 5% or 8% 2-propanol in TMP. Quantitation was made by determining the quantity of standard required to produce the same peak height as obtained in the spiked sample.

RESULTS AND DISCUSSION

Figure 1 shows a chromatogram of a mixture of the standard compounds detected at 254 and 280 nm. It can be seen that both carbofuran and its 3-hydroxy metabolite are about sevenfold more sensitive at 280 nm than at 254 nm. These results are similar to other published data comparing the two wavelengths for these compounds (Sparacino and Hines, 1976). However, 3-ketocarbofuran is more than 30 times more sensitive at 254 nm than at 280 nm. Thus, for the present work 280 nm was used for carbofuran and 3-hydroxycarbofuran determinations while 254 was employed for 3-ketocarbofuran. Minimum detectable quantities taken at a 3:1 signal:noise ratio were 1 ng of carbofuran, 0.4 ng of 3-ketocarbofuran, and 3.0 ng of 3-hydroxycarbofuran. Usually five–ten times these levels were injected for quantitative measurements. The
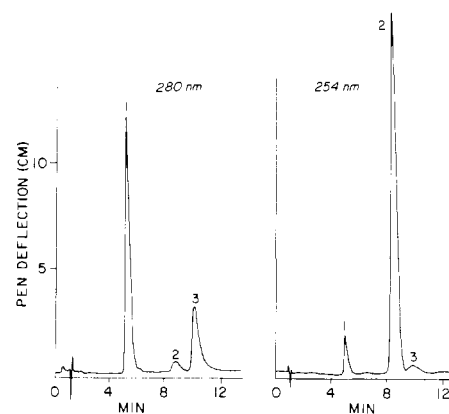

Figure 1. Chromatograms of a mixture of 200 ng of carbofuran (1), 135 ng of 3-ketocarbofuran (2), and 200 ng of 3-hydroxycarbofuran (3) at 254 and 280 nm detection. Mobile phase, 5% 2-propanol in TMP; sensitivity, 0.01 absorbance units full-scale.

response was linear over a range extending from the detection limit up to more than 100 ng injected for all three carbamates. Responses and retention times were reproducible from one day to the next.

The extraction and clean-up procedure was similar to that described earlier (Lawrence, 1976a,b) but optimized for carbofuran and its two metabolites. The extraction was carried out without the aid of the acid hydrolysis step as described by Cook et al. (1969). Thus, the method described herein only is applicable to carbofuran plus the unconjugated forms of the two metabolites. For analysis of the conjugated metabolites, which can often constitute as much as 97% of total metabolites present (Dorough, 1968; Knaak et al., 1970), the method of Cook et al. (1969) or the extraction described by Van Middelem and Peplow (1973) will probably be required. Since both include acid hydrolysis, a significant cleanup afterwards usually is required for analysis by GLC. We have not yet applied these extraction techniques to HPLC analysis, although it would be expected that a rigorous cleanup would also be required. The use of hydrolytic enzymes such as glucosidase to release the conjugated metabolites might prove to be superior to acid hydrolysis. Also HPLC analysis of the conjugate itself after perbenzoylation may be useful since hydrolysis would be avoided. Perbenzoylation of glycoside conjugates for HPLC analysis has been reported (Nachtmann et al., 1976; Suzuki et al., 1976; Ullman and McCluer, 1977).

The Florisil cleanup was adequate for determination of pesticide and metabolite concentrations down to 0.05 ppm. The 3-hydroxycarbofuran fraction was slightly cleaner than the fraction containing the other two. The elution patterns were very consistent from one day to the next as well as between different Florisil batches.

Figure 2 shows the simultaneous dual-wavelength detection of carbofuran and 3-ketocarbofuran in carrot spiked at 1.0 ppm each. While 3-ketocarbofuran was easily detected at 254 nm, there appeared earlier a large peak which completely masked the carbofuran. This peak was absent from the sample blank when run at 280 nm (as shown in the lower chromatogram as background under the cross-hatched peak of carbofuran). The longer wavelength was often more selective than 254 nm and resulted in a lower degree of interfering peaks. For example, cabbage samples could not be analyzed for 3-hydroxycarbofuran at 254 nm because of large off-scale peaks which interfered. These were absent at 280 nm. The second chromatogram in Figure 2 is an example of an analysis of a field-treated turnip sample for carbofuran and 3-ketocarbofuran. The
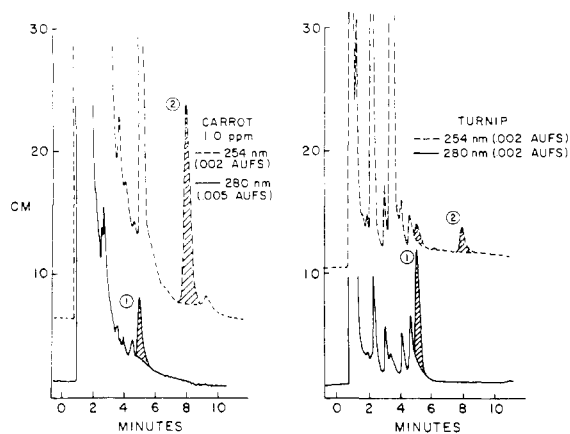
Figure 2. Chromatograms of carbofuran (1) and 3-ketocarbofuran (2) in carrot spiked at 1.0 ppm each, and a sample of field-treated turnip (50 mg of each injected). Mobile phase, 5% 2-propanol in TMP, 1.0 mL/min. The cross-hatched peaks represent the differences obtained between blank and spiked samples (i.e., peaks due to the presence of the carbamates).
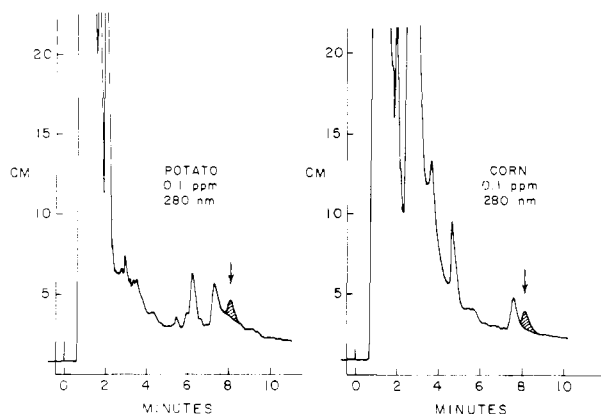

Figure 3. Chromatograms of potato and corn spiked at 0.1 ppm carbofuran (70 mg of sample injected). Mobile phase, 3% 2-propanol in TMP, 1.0 mL/min; sensitivity, 0.002 AUFS.

high level of carbofuran (1.13 ppm) found and the reasonably clean chromatogram enabled detection at 254 nm as well as 280 nm. However, the latter results are much superior for quantitation. The 3-ketocarbofuran was present at 0.095 ppm as detected at 254 nm.

Figure 3 shows chromatograms obtained from samples of potato and corn at 0.1 ppm each carbofuran with 280 nm detection. The solvent polarity was reduced from 5 to 3% 2-propanol in TMP in order to remove interfering peaks at these low levels. Carbofuran and 3-hydroxy-carbofuran could not be detected at 254 nm at a level of 0.1 ppm in any of the samples analyzed. The chromatograms of Figure 3 show results which are considered to be near the detection limit of the method. Although 280 nm was more sensitive to carbofuran and 3-hydroxy-carbofuran, the detector noise was about fourfold greater than at 254 nm, thus preventing the use of higher sensitivity settings than 0.002 AUFS. The noise increase can be partially attributed to the phosphor screen required to convert 254 nm light to 280 nm. This wavelength was found to be the noisiest of all the wavelengths available for the Model 440 detector. Much of the high frequency noise was eliminated by electronic damping (gain control) of the recorder but not to an extent where recorder linearity was affected. The problem can be alleviated somewhat by injecting larger quantities (e.g., 200–300 mg of equivalent sample), but overloading resulting poorer separations and shorter column life might result. We prefer to work at high sensitivity and to inject as little
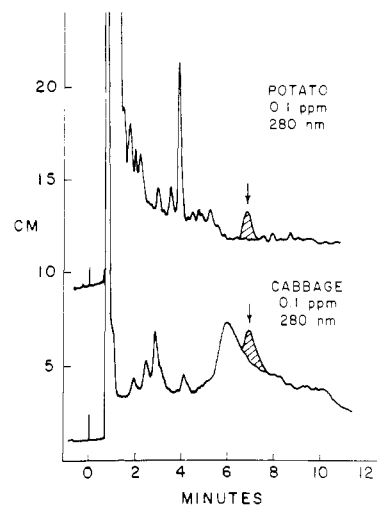

Figure 4. Chromatograms of potato and cabbage spiked at 0.1 ppm each of 3-hydroxycarbofuran (135 mg of each sample injected, 0.002 AUFS). Mobile phase, 8% 2-propanol in TMP, 1.0 mL/min.

sample material as possible. This has enabled us to use columns regularly for residue analysis for 1–2 years with little deterioration. Only occasional washings with methanol and acetone were required to remove any build up of polar contamination. The Florisil column cleanup removed most of the very polar contaminants which might otherwise irreversibly contaminate the HPLC column.

Figure 4 shows chromatograms of 3-hydroxycarbofuran spiked in potato and cabbage at 0.1 ppm. These also illustrate results near the detection limit of the method, although in some foods such as potatoes, peas, and corn the extracts were clean enough to permit the injection of larger volumes of extract to permit detection of lower levels of the carbamate.

Recovery studies carried out on the carbamates in the foods studied at 1.0 and 0.1 ppm varied from 75–110% for carbofuran (average 82%), 68–100% for 3-ketocarbofuran (average 82%), and 82–100% for 3-hydroxycarbofuran (average 94%).

The described extraction and clean-up technique is a modification of a method for GLC analysis of pesticides (Lawrence and McLeod, 1977). This illustrates that HPLC analyses can be directly integrated into existing GLC methodology for the determination of sub-ppm concentrations of many pesticides. It has been shown earlier (Lawrence, 1976b) that HPLC compliments GLC in many respects for direct pesticide analysis using the same extraction and clean-up procedure for both detection systems.

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Structural Elucidation of an Octachloro Component of Technical Chlordane (Compound K) by Spectroscopic and X-Ray Methods

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The technical chlordane constituent designated compound K is a formal isomer of chlordane ($C_{10}H_6Cl_8$) which is formed by chlorination of α-chlordene (1) ($C_{10}H_6Cl_6$) via a Wagner–Meerwein rearrangement. Structural elucidation of K involves dechlorination reactions, especially spectroscopic studies (MS, ^1H NMR, and ^{13}C NMR) of various photodechlorination products (3–7). From these data a structure for compound K is proposed. Confirmation of this structure is obtained by single-crystal x-ray diffraction. According to the results, compound K corresponds to 2,4,4,5,6,6,7,8-octachlorooctahydro-1,5-etheno-pentalene (2).

The pesticide technical chlordane is a multicomponent mixture of chlorinated hydrocarbons which is formed by chlorination of the Diels–Alder adduct of hexachloro-cyclopentadiene and cyclopentadiene. Only a few components of this mixture are well characterized. These are chlordene, heptachlor, cis- and trans-chlordane, cis- and trans-nonachlor (Velsicol Chemical Corp., 1971), four isomeric chlordenes designated α-, β-, and γ-chlordene (Gäb et al., 1975, 1976; Cochrane et al., 1975; Wilson and Sovocool, 1977), and chlordene-C (Gäb et al., 1977). α-Chlordene can add chlorine to give a formal isomer of chlordane ($C_{10}H_6Cl_8$), which appears as peak K (referred to as compound K) in the gas chromatogram of technical chlordane (Polen, 1966).

Recently, Wilson and Sovocool (1977) reported on the structure of compound K. Four possible alternatives (I, II, III, IV) were discussed (Figure 1). Although structure IV seemed to be most consistent with the spectroscopic data no unequivocal verification of this structure could be obtained. It was suggested that compound K is 2,4,5,5,6,7,8,8-octachloro-2,3,3a,4,5,7a-hexahydro-1,4-methano-1H-idene. The present study was undertaken to examine the proposed structures by means of spectroscopic analysis of compound K and its photodechlorination products as well as by x-ray diffraction.

EXPERIMENTAL SECTION

Chromatography. A Packard 417 gas chromatograph was employed for both qualitative and quantitative purposes. A glass column (diameter, 3 mm; length, 2 m) packed with 5% QF-1 on Chromosorb W-AW-DMCS (80–100 mesh) was used. Temperatures were: inlet, 250 °C; detector, 300 °C; column, 180 °C. The nitrogen flow rate was 30 mL/min. Column chromatography was carried out with silica gel from Merck, Darmstadt (grain size

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0.2–0.06 mm) as adsorbent, and n-hexane as eluent. For thin-layer chromatography silica gel 60 TLC plates with a layer thickness of 0.25 mm (Merck, Darmstadt) were used, with n-hexane as developing solvent. After developing plates were sprayed with 1% diphenylamine solution and irradiated with UV light for about 5 min (λ 254 nm) to detect substances.

Table I gives the respective TLC and GLC characteristics of compounds (1–7).

Chlorination of α-Chlordene (1). α-Chlordene (1) (3.0 g) was suspended in 100 mL of CCl_4 and treated with Cl_2 at 60 °C for 1 h. After cooling, excess chlorine was driven off with N_2 and the solvent in the remaining clear solution was removed under reduced pressure. The oily residue was subjected to column chromatography on 150 g of silica gel and eluted with n-hexane. Crystallizations from the eluates yielded 2.75 g of compound K (2).

Reduction of Compound K (2) with $CrCl_2$. Compound K (2) (0.45 g) was dissolved in 80 mL of acetone. Aqueous $CrCl_2$ (30 mL) (Fisher Scientific) was added under nitrogen, and the mixture was refluxed for 3 h. The reaction mixture was cooled, diluted with 500 mL of distilled water, and extracted with benzene. The combined extracts were dried and evaporated. The crystalline residue (0.33 g) consisted of α-chlordene and mono-dechloro-α-chlordene [from dechlorination at the allylic position in (1)] in the ratio 1.8:1.

UV Dechlorination of Compound K (2). In a typical UV irradiation procedure, 0.5 g of compound K (2) was dissolved in 80 mL of n-hexane. A quartz water-jacketed cooling finger containing a Hg high-pressure lamp (HPK 125 W Philips, with wavelengths above 230 nm) was immersed in the solution. After an irradiation time of 35 (105) min, dechlorination products were determined by GC analysis in the following quantities: 14.8% (6.5%) of **3**, 51.0% (34.8%) of **4**, 21.4% (36.3%) of **5**, 2.2% (8.3%) of **6**, and <1% (3.5%) of **7**. Repeated irradiations were performed when additional quantities of minor photoproducts were required. Separation of the products was carried out on a 100-g silica gel column which was eluted with n-hexane.

Spectroscopy. Infrared spectra were recorded as KBr pellets on a Perkin-Elmer Model 577 grating spectrometer.