Identification of Three Neutral Contaminants in Production Grade 2,4-D

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The structure of a major neutral contaminant in production grade 2,4-D is derived by chemical and spectroscopic data and confirmed by synthesis. Two other neutral impurities are tentatively identified and shown to be isomeric with the major neutral contaminant by combined glc–mass spectroscopy. Evidence is presented to show that these impurities can interfere with the glc analysis of 2,4-D for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin content.

Although the toxicology of pesticides is extensively studied, little research has been devoted to the nature and toxicological significance of trace amounts of impurities in pesticide chemicals. However, the observation (Courtney *et al.*, 1970) that samples of 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) used in a teratogenic study contained significant amounts of the highly toxic compound 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (Higginbotham *et al.*, 1968) has led to an increased interest in the nature of impurities in pesticides.

Recently, we had occasion to analyze, by gas–liquid chromatography, the neutral extracts of some samples of production grade 2,4,5-T and 2,4-D (2,4-dichlorophenoxyacetic acid). The glc tracings of the extracts from both pesticides indicated the presence of several neutral contaminants and in the case of the 2,4-D extract three of these impurities had very similar retention times to that of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. The present paper describes the isolation and characterization of the major of these three impurities and the tentative identification of the other two. Furthermore, this work points out the need for caution on the part of the analyst in interpreting glc data alone when analyzing samples of pesticides for impurities.

EXPERIMENTAL

Materials. All solvents were pesticide grade and used as received. Production grade 2,4-D and the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin were generously supplied by Dow Chemical Company, Midland, Michigan. 2,6-Dichlorophenol, 2,4-dichlorophenol, and methylene iodide were obtained from Aldrich Chemical Company, Eastman Organic Chemicals, and Fisher Scientific, respectively.

Instruments. Mass spectra were obtained with a Hitachi Perkin-Elmer (RMS-4) mass spectrometer coupled with a Perkin-Elmer (Model 990) gas chromatograph, fitted with flame ionization detectors. The stainless steel column, 6-ft × $^1/_8$-in. o.d., was packed with 3% XE-60 on 100–120 mesh Chromosorb W (HP), Chromatographic Specialties Ltd. Operating conditions were: detector and injector temperatures, 220°C; oven temperature, 200°C; carrier gas, helium at a flow rate of 34 ml/min. Nmr spectra were obtained with a Varian A-60A spectrometer in deuterochloroform solution with tetramethylsilane as internal standard. Ultraviolet spectra were obtained with a Unicam model SP 1800 spectrometer. Melting points were determined with an Electrothermal capillary melting point apparatus and are uncorrected. Microanalyses were performed by A. B. Gygli, Microanalysis Laboratories Limited, Toronto, Ontario.

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Chromatography. Baker analyzed reagent silica gel was used for column chromatography. Preparative thin-layer chromatography was performed on plates coated with 0.5 mm silica gel (MN-Silica Gel G/u.v.$_{254}$, Macheney, Nagel & Co.). A mixture of hexane–benzene (4:1) was used as developing solvent. Glc analyses were obtained on several instruments using the columns and conditions listed in Table I.

Extraction of 2,4-D Neutral Impurities. Production grade 2,4-D (1 kg) was extracted by shaking with hexane (1 l.) and filtering through a medium porosity sintered glass funnel. The extraction process was repeated with four additional (1 l.) portions of hexane. A total of 8 kg of 2,4-D was processed in this manner and the combined extracts were evaporated to dryness on a rotary evaporator at 40°C. The residue was taken up in ether (100 ml) and extracted with two 50-ml portions of 10% sodium hydroxide solution, washed with water (50 ml), and the ether layer was dried with anhydrous sodium sulfate. Evaporation of the ether gave the neutral impurities as a pale yellow semi-solid.

Purification of Impurity III. The neutral residue was dissolved in a minimum amount of hexane and chromatographed on a column of silica gel (1.5 cm × 42 cm). Elution with hexane (200 ml) gave a fraction that contained impurity III as the major component as indicated by glc. Preparative tlc of this fraction gave a band (R_f 0.62) which was eluted with ether and shown by glc to contain impurity III with a trace of impurity II. Three recrystallizations of this material from hexane gave pure III as colorless crystals, mp 101–101.5°C: nmr δ 5.76 (s, 2 H), 7.25–7.41 (m, 6 H); uv λ_{max}^{EtOH} 228 nm (ϵ 19,900), 281 (2740), 289 nm (2240); mass spectrum *m/e* 336 (parent ion).

Anal. Calcd. for $C_{13}H_8O_2Cl_4$: C, 46.42; H, 2.38; Cl, 42.26. Found: C, 46.34; H, 2.35; Cl, 41.24.

Synthesis of Bis(2,4-dichlorophenoxy)methane and Bis-(2,6-dichlorophenoxy)methane (3 and 5). The appropriate dichlorophenol (16.3 g, 0.1 mol), anhydrous potassium carbonate (14 g, 0.1 mol), methylene iodide (4 ml, 0.05 mol) and dry acetone (100 ml) were combined and refluxed for 8 hr. The mixture was cooled, filtered, and the solvent removed *in vacuo*. The residue was dissolved in methylene chloride (100 ml), washed with two 75-ml portions of 10% sodium hydroxide solution, one 75-ml portion of water, and dried over anhydrous sodium sulfate. Evaporation of the solvent gave a pale yellow solid which was recrystallized twice from 95% ethanol to give colorless crystals of **3** and/or **5**. Bis(2,4-dichlorophenoxy)methane, mp 100–102°C [lit. mp 100.4–100.8°C (Miron and Lowy, 1951)]. Mixture melting point with III 101–102°C; nmr δ 5.75 (s, 2 H), 7.25–7.41 (m, 6 H); mass spectrum *m/e* 336 (parent ion). Bis(2,6-dichlorophenoxy)methane, mp 78.5–80°C; nmr δ 5.68 (s, 2 H), 6.85–740 (m, 6 H); mass spectrum *m/e* 336 (parent ion).

Table I. Glc Parameters

Column	Stationary phases	Support	Dimensions	Injector/ detector temperature, °C	Oven temperatures, °C	Detection	Carrier gas flow rate, ml/min
Glass	QF-1/SE-30 6%/4%	80–100 mesh Chromosorb W (HP)	5-ft × 1/4-in. (o.d.)	225/210	190	Tritium foil[c] electron capture	120[a]
Glass	SE-52 2.5%	80–100 mesh Gas Chrom Q	3-ft × 1/8-in. (o.d.)	220/220	185	Tritium foil[d] electron capture	60[a]
Stainless steel	SE-30 5%	80-100 mesh Chromosorb W	6-ft × 1/8-in. (o.d.)	230/250	200	Flame ionization[e]	30[a]
Stainless steel	XE-60 3%	100–120 mesh Chromosorb W (HP)	6-ft × 1/8-in. (o.d.)	220/220	200	Flame ionization[f]	35[b]

[a] Carrier gas, nitrogen. [b] Carrier gas, helium. [c] Varian Aerograph Model 600-D. [d] Varian Aerograph Model 2100. [e] Hewlett-Packard Model 700. [f] Perkin-Elmer Model 990.

Table II. Gas Chromatographic Comparison of Three 2,4-D Neutral Impurities and 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin

Column	Retention time, min 2,3,7,8-tetrachlorodioxin	2,4-D Impurities I	II	III
QF-1/SE-30	11.5	9.2	10.4	11.6
SE-52	7.4	5.6	6.5	7.4
SE-30	7.9	6.0	6.7	7.7
XE-60	5.8	5.7	6.3	7.6

Anal. Calcd. for $C_{13}H_8O_2Cl_2$: C, 46.42; H, 2.38. Found: C, 46.56; H, 2.41.

Synthesis of 2,2′,4,6′-Tetrachlorodiphenoxymethane (6). α-2,4-Trichloroanisole (2 g, 0.01 mol) [prepared according to the method of Lashua and Ranck (1966)] was stirred and refluxed for 8 hr with a suspension of the potassium salt of 2,6-dichlorophenol in dry benzene. The cooled mixture was washed with water and the benzene layer dried over anhydrous sodium sulfate. Evaporation of the solvent gave a colorless solid which, upon four recrystallizations from 95% ethanol, gave colorless needles, mp 85–86°C: nmr δ 5.75 (s, 2 H), 6.88–7.61 (m, 6 H); mass spectrum *m/e* 336 (parent ion).

Anal. Calcd. for $C_{13}H_8O_2Cl_4$: C, 46.42; H, 2.38. Found: C, 46.53; H. 2.48.

RESULTS AND DISCUSSION

The analysis of the neutral extracts of production grade 2,4-D was investigated by glc using the columns and conditions listed in Table I. It is apparent, from an examination of Figure 1, that several neutral impurities are present and, as indicated in Table II, three of these components, labeled I, II, and III in Figure 1, have retention times similar to that of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin under the glc conditions stated in Table I.

Isolation and Characterization of Component III. Since component III appeared to be present in relatively substantial quantities, it was decided to isolate and identify this material. Hexane extraction of production grade 2,4-D followed by column chromatography, on silica gel, gave a fraction enriched in component III as estimated by glc. Further purification of this fraction by preparative thin-layer chromatography, on silica gel, gave a pale yellow solid which, after repeated recrystallizations from hexane, gave pure III as colorless crystals, mp 101–101.5°C. The mass spectrum of III shows a parent ion at *m/e* 336 and an isotopic distribution pattern typical of four chlorine atoms (McLafferty, 1967). Consideration of this data together with the elemental analysis (experimental) gives rise to $C_{13}H_8O_2Cl_4$ as a probable molecular formula for compound III. The ultraviolet spectrum of III exhibits absorption bands at 228 nm (ε = 19,900), 281 nm (ε = 2740) and 289 nm (ε = 2240), suggesting a substituted phenol ether (Scott, 1964). The nuclear magnetic resonance spectrum of III consists of a complex multiplet between 7.25 and 7.41 ppm (6 H) and a singlet at 5.76 ppm (2 H), indicating the presence of six aromatic protons and a substituted methylene group. These facts suggest two possible part structures **1** and **2** for compound III.

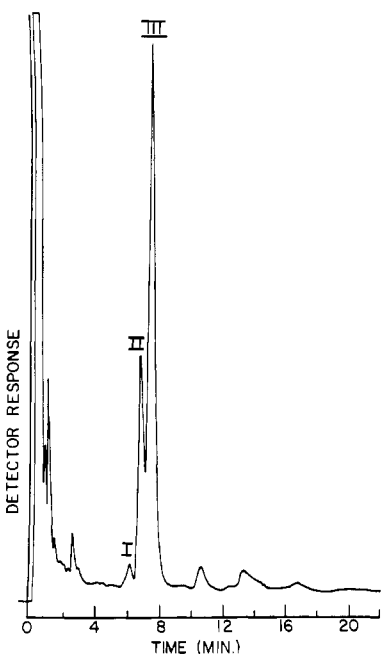
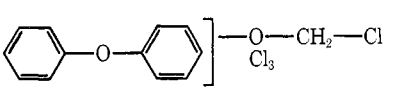


Figure 1. Chromatogram of 2,4-D neutral extract. Column 6′SE-30; temperature, 200°C; nitrogen flow rate 30 ml/min



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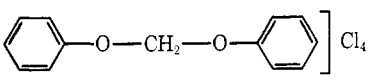

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A choice between part structures **1** and **2** was made when it was observed that an acetone solution of III did not react with aqueous silver nitrate. Since part structure **1** contains an ionizable halide function it would be expected to give a precipitate with silver nitrate. Part structure **2**, on the other hand, contains only aromatic halogen and would not be

Table III. Comparison of Physical Properties of 2,4-D Impurities and Synthetic Diphenoxymethanes

	Compound					
	I	Bis-(2,6-dichlorophenoxy) methane	II	2,2',4,6'-Tetrachlorodiphenoxymethane	III	Bis-(2,4-dichlorophenoxy)-methane
Retention time, min	6.0,[a] 9.2[b]	6.0,[a] 9.2[b]	6.7,[a] 10.4[b]	6.7,[a] 10.4[b]	7.7,[a] 11.6[b]	7.7,[a] 11.6[b]
Mass spectrum (parent ion and main fragmentation ion) amu	336 175	336 175	336 175	336 175	336 175	336 175
nmr, ppm	...	...	...	...	5.76 (2 H) 7.25–7.41 (6 H)	5.75 (2 H) 7.25–7.41 (6 H)
Melting point, °C	...	78.5–80	...	85–86	101–101.5	100–102

[a] SE-30 column. [b] SE-30/QF-1 column.

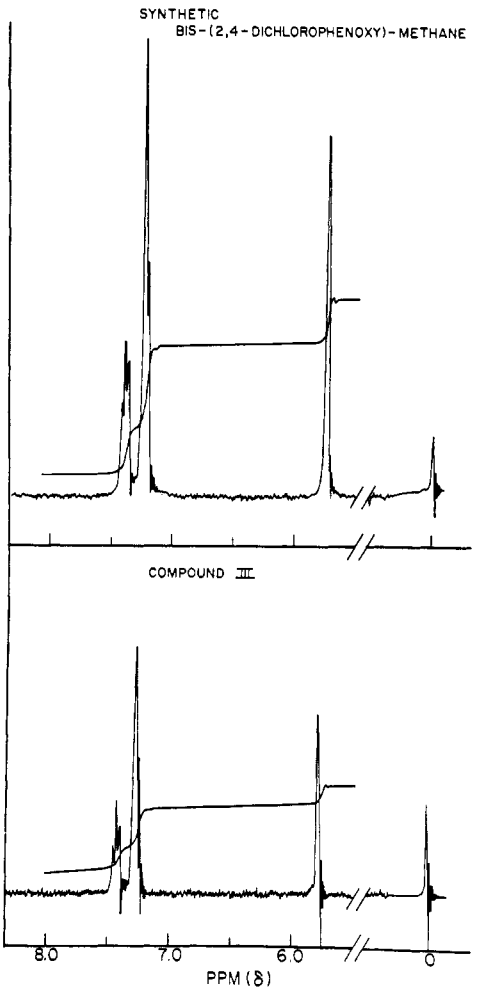


Figure 2. Nmr spectra of compound III and synthetic bis(2,4-dichlorophenoxy)methane

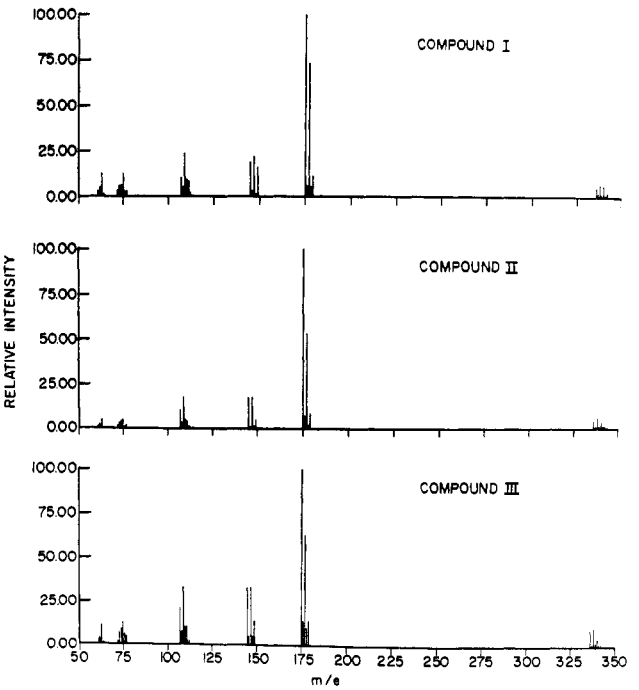
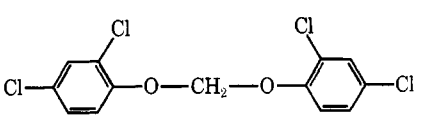


Figure 3. Mass spectra of compounds I, II, and III

expected to react with silver nitrate. Thus, part structure **2** was favored for compound III and furthermore, since the most reasonable precursor of III is 2,4-dichlorophenol, structure **3** could be written for compound III.



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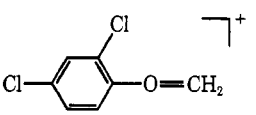
The mass spectrum of compound III supports structure **3** since the main fragmentation ion at *m/e* 175 represents the loss of $C_6H_3OCl_2$ from $C_{13}H_8O_2Cl_4$ and can be readily accommodated by cleavage of one of the O–CH_2 bonds in **3** to give the ion **4**, analogous to the loss of an alkoxyl group of a ketal (Budzikiewicz *et al.*, 1967).



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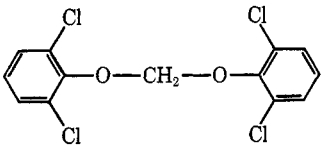
Structure **3** was proven to be correct by synthesis of bis-(2,4-dichlorophenoxy)methane, and its identity with III confirmed by mixture melting point, glc retention time, and comparison of nmr spectra (Figure 2) and mass spectra (Table III).

The Identity of Impurities I and II. Since impurities I and II are present to a lesser extent than III, as indicated by glc, no attempt was made to isolate them; instead combined glc–mass spectroscopy was used to elucidate their structures. The mass spectra of impurities I, II, and III are virtually identical (Figure 3), suggesting that they are positional isomers. Furthermore, since it is known that production grade 2,4-D (Lot #092440) contains small amounts of 2,6-dichlorophenol as an impurity (Wiffen, 1970), it is reasonable to assume that compounds I and II might have structures **5**

and **6**. Structures **5** and **6** were synthesized and shown to be identical to I and II, respectively, by comparison of glc retention time, on two columns and mass spectra (Table III).



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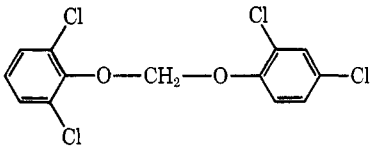

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Semiquantitative analysis of the commercial sample of 2,4-D used in this study indicated that compounds I, II, and III were present at levels of 1, 10, and 30 ppm, respectively. The toxicological significance of these three compounds as impurities in production grade 2,4-D is not known. However, from a study of the teratogenic effects of both production grade 2,4-D and purified 2,4-D (Khera, 1972), it appears that compounds I, II, and III have no adverse effects at the levels administered in his investigation.

ACKNOWLEDGMENT

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Varietal Differences and Seasonal Effects on Fatty Acid Composition and Stability of Oil from 82 Peanut Genotypes

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Eighty-two peanut genotypes of diverse genetic background were examined over a 3-year period for varietal differences and seasonal effects on fatty acid composition and oil stability (autoxidation induction period). The range in oil stability among genotypes was 11.6 to 18.5 days and the ranges in fatty acid values were: 7.4 to 12.9% palmitic; 1.6 to 5.3% stearic; 35.7 to 68.5% oleic; 14.1 to 40.3% linoleic; 0.9 to 2.2% arachidic; 0.6 to 2.0% eicosenoic; 1.3 to 5.1% behenic; and 0.6 to 2.0% lignoceric acid. Yearly mean fatty acid values for all varieties showed relatively small but significant ($p < 0.01$) yearly variations in fatty acid composition. Yearly variations in oil stability values were large and could not be accounted for by yearly variations in fatty acid composition. Simple regression of oil stability on various fatty acids or combinations thereof showed significant correlations within a given year but with wide variations in magnitude of r^2 and estimated regression coefficients among years.

It has been shown that oils obtained from different botanical types of peanuts (*Arachis hypogaea* L.) differ considerably in tendency to develop oxidative rancidity and that this tendency is related, at least in part, to the content of linoleic acid (Fore *et al.*, 1953; Higgins and Holley, 1951; Holley and Hammons, 1968). Crawford and Hilditch (1950) called attention to the differences in oleic and linoleic acid content of peanut oil from different sources and suggested that these differences should be reflected in oil stability. In consideration of these differences and their probable bearing on liability to oxidative rancidity, Crawford and Hilditch (1950) advocated the commercial production of varieties low in linoleic acid. In an investigation of the relationship between raw peanut oil composition and stability, Fore *et al.* (1953) found linoleic acid to be a factor in the development of oxidative rancidity but were unable to explain all observed differences in stability on the basis of either linoleic acid or tocopherol content. The stability values obtained by Fore *et al.* (1953) were approximately twice those reported earlier (Fisher *et al.*, 1947) for a freshly refined, bleached, and deodorized oil of similar linoleic acid and tocopherol content. As a result of these and other observations, Fore *et al.* (1953) suggested that the rate of autoxidation of crude peanut oil is influenced by antioxidants and/or synergists other than tocopherols and that these components are removed in refining.

Holley and Hammons (1968) reported a correlation of −0.92 between linoleic acid content and oil stability. This

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