woody) when nootkatone provided the dominant flavor note.

The panel was more sensitive to subtle differences between the oils when evaluated on the basis of aroma than on the basis of flavor in grapefruit juice. One factor that should be considered in determining the threshold of nootkatone in grapefruit juice is the level of nootkatone present in the juice prior to addition of the flavor fraction containing nootkatone. MacLeod and Buigues (1964) isolated 77 mg of crude nootkatone from 1 gal of freshly extracted peel-oil-free juice. This amount represents ~20 ppm of nootkatone in the peel-oil-free juice. Berry et al. (1967) noted that grapefruit juice containing 0.005% oil would contain an average of less than 0.5 ppm of nootkatone, but they considered only nootkatone present in the added peel oil. We used thin-layer chromatography (TLC) to estimate a nootkatone content of ~7 ppm in our single-strength grapefruit juice sample prior to addition of any oil (Tatum and Berry, 1975). This level is slightly above the reported threshold in grapefruit juice and is within the range of 6–7 ppm of nootkatone Berry et al. (1967) considered optimum in grapefruit juice. In Table I the highest level of nootkatone contributed to single-strength juice by the addition of grapefruit oil was only 0.8 ppm. Thus, variations in nootkatone levels in juices containing the high- and low-nootkatone oils represented only slight changes in above-threshold levels. It is perhaps not surprising that the flavor panel was generally unable to distinguish variations in nootkatone content due to the oils added to juice. This situation is comparable to one that exists commercially when cold-pressed grapefruit oil is added to frozen concentrated grapefruit juice, since considerable nootkatone is undoubtedly present in the frozen concentrated juice prior to addition of oil and other flavor fractions.

Although nootkatone is considered the primary flavor agent in grapefruit (MacLeod, 1966), it is clear that other consituents of the oil modify the flavor of this agent at above-threshold levels. Aldehydes are also important to the flavor and aroma of grapefruit oil, and Kesterson et al. (1971) have indicated that oils with maximum total aldehydes content (~1.8%) and moderately high nootkatone content (0.5–0.7%) are the preferred oils by organoleptic evaluations. Other unidentified components, probably similar to nootkatone in structure, are present in crude nootkatone isolated from grapefruit oil, and they contribute to grapefruit flavor (Stevens et al., 1970). They, like nootkatone, are probably present in relatively high levels in frozen concentrated juice and in deoiled single-strength juice. Several esters found in grapefruit oil have been suggested—but, as yet, not established—as contributors to grapefruit flavor (Moshonas, 1971).

Nootkatone, in combination with several other carbonyl-containing constituents, is probably responsible for the flavor of good-quality grapefruit oil. However, nootkatone, by itself, may not provide an adequate grapefruit flavor in foods.

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Synthesis of Nonachloro-2-phenoxyphenol

Nonachloro-2-phenoxyphenol (I) was prepared by reduction of 2,3,4,5,6-pentachloro-4-(pentachlorophenoxy)-2,5-cyclohexadienone (II) with sodium iodide in methanol–chloroform solution. A combination of chromatographic procedures was used to remove impurities from I.

Approximately 50 million lb of technical pentachlorophenol (PCP) is manufactured in the United States annually. Most of the PCP is used in the wood products industry for insect, fungus, and slime control (Bevenue and Beckman, 1967). The remainder is used in agriculture and other industries. Analysis of technical PCP shows there are numerous chlorinated byproducts present in fairly high concentrations (Schwetz et al., 1974). These byproducts arise during the manufacture of PCP and include hexachloro-2,5-cyclohexadienone (Kulka, 1961; Wilkinson, 1975), polychlorodibenzo-*p*-dioxins (Jensen and Renberg, 1972; Firestone et al., 1972; Plimmer et al., 1973; Buser, 1975), polychlorodibenzofurans (Firestone et al., 1972; Buser and Bosshardt, 1976), polychlorodiphenyl ethers

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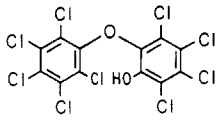
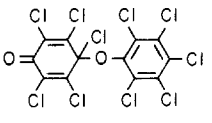
(Firestone et al., 1972), and polychlorophenoxyphenols (Rappe and Nilsson, 1972; Jensen and Renberg, 1972; Nilsson and Renberg, 1974; Deinzer et al., 1978, 1979a). The extremely toxic 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) has not been detected in PCP (Ahlborg and Thunberg, 1980) nor is there any prima facie reason why it should be. The toxicity of some of these contaminants as well as that of PCP itself has caused the EPA to place technical PCP under review in the RPAR (rebuttable presumption against registration) program in order to determine whether its use should be continued, restricted, or banned.

The biological properties of the chlorinated phenoxyphenols, which constitute up to 8% of technical PCP preparations (Deinzer et al., 1979b), have only recently come under investigation, and indications are that more information about these compounds is urgently needed. Thus, in recent studies with human erythrocytes in vitro, the three nonachlorophenoxyphenols have been shown (Miller and Deinzer, 1980) to be 110–250 times more hemolytically active than pure PCP. Our objective was to synthesize and purify a sufficient quantity of nonachloro-2-phenoxyphenol (I) to carry out toxicity studies.



I



II

The synthesis of this product from 2,3,4,5,6-pentachloro-4-(pentachlorophenoxy)-2,5-cyclohexadienone (II) has been described (Denivelle et al., 1959). In this report II was rearranged thermally in the presence of ferric chloride to 3,4,4,5,6-pentachloro-2-(pentachlorophenoxy)-2,5-cyclohexadienone and then reduced. Our results show that II may be converted to I by treatment with sodium iodide in methanol–chloroform at room temperature. We report these results and the methods we used to purify I.

EXPERIMENTAL SECTION

Gas chromatography–mass spectrometry analyses were carried out on a Finnigan 4023 instrument. A Pyrex gas chromatography column (0.32 cm × 4.88 m) packed with 3% OV-101 on 80–100-mesh high-performance Chromsorb W (Johns-Manville Co.) was used for separation. Proton NMR spectra were obtained on a Varian 100 spectrometer and carbon-13 NMR spectra on a Varian CFT-80 instrument. Infrared measurements were made on a Perkin-Elmer 457 instrument. Melting points are uncorrected and were determined on a Fisher-Johns melting point apparatus. Analyses by high-pressure liquid chromatography (LC) were carried out on either a Waters Model ALC 202 instrument or a Spectrophysics 8000 A instrument, using a 4.0 × 300 mm Waters Associates μBondapak C_{18} column or a 4.6 × 250 mm Licrosorb Si 60 5μ column.

Synthesis. 2,3,4,5,6-Pentachloro-4-(pentachlorophenoxy)-2,5-cyclohexadienone (II) was prepared from PCP (106.4 g, 0.40 mol) according to the method of Reed (1958). The product was recrystallized from hot carbon tetrachloride: yield, 75.2 g (71.5%); mp 174–175 °C [lit. mp 177–178 °C (Reed, 1958)]; IR ν 1688, 1577 cm^{-1} [lit. IR 1689, 1577 cm^{-1} (Reed 1958)].

Typically, the dienone II (1.0 g, 11.4 mmol) was dissolved in 825 mL of carbon tetrachloride. A solution of 500 mg of sodium iodide in 150 mL of methanol–chloroform (1:1) was added dropwise over a period of 1 h under constant stirring in the dark. The reaction mixture was stirred at room temperature for an additional period of 24–48 h. The solvent was removed from the product mixture in vacuo by using a rotary evaporator. The yield of I in the residue was 294 mg (31.4%) as measured by high-pressure liquid chromatography (LC) analysis. Because of the large volumes of solvent involved in the reduction, it was inconvenient to scale up the procedure. Consequently, six 1.0-g batches of II were reduced, and the isolated products combined for subsequent purification.

Purification. *Ion-Exchange Chromatography.* The combined product from the reduction of II (6 g) was dissolved in 50 mL of methanol and 5 mL of methylene chloride and pumped at 1–2 mL/min onto a 2.5 × 33 cm Dowex 21K (50–100-mesh) ion-exchange column. After addition of the sample to the column, the following series of eluants was used at a flow rate of 6–7 mL/min: (1) 50 mL of methanol; (2) 1500 mL of 1.5 N acetic acid in methanol; (3) 250 mL of 1.75 N acetic acid in methanol; (4) 1200 mL of 2.0 N acetic acid in methanol; (5) 1300 mL of 4.0 N acetic acid in methanol. The desired product was eluted with solvent system 4, i.e., with 2.0 N acetic acid in methanol. The elution of the product was monitored with a Gilson Model HM detector set at 280 nm. A heart cut of the emerging peak was taken, which when concentrated to dryness in vacuo yielded 539 mg of I. Edge fractions of the peak were also collected and concentrated to dryness to yield 537 mg of residue. The residue from the edge fractions was rechromatographed, yielding 390 mg of product: yield, 1.02 g (18% based on II). The product when chromatographed on the high-pressure liquid chromatograph with a 254-nm detector wavelength setting showed peak areas for impurities whose sum was <5% of the total area for I.

A portion of the above product (300 mg), containing I, was dissolved in 40 mL of 0.06 N sodium hydroxide and 25 mL of methanol. This solution was extracted 7 times with 25-mL portions of trimethylpentane. The aqueous fraction was acidified with dilute sulfuric acid to pH 1–2 and extracted with five 25-mL portions of methylene chloride. The combined organic phase was dried over anhydrous sodium sulfate and the solvent was removed in vacuo. At this stage the product, when chromatographed on the high-pressure liquid chromatograph with a 254-nm detector wavelength setting, showed a combined peak area due to impurities of <1% of the total peak area of I.

Reverse-Phase Chromatography. Typically, 50 mg of product (I) from the previous step in methanol was added to a 2.2 × 15 cm column containing 25 g of RP-18 (25–40 μm of EM-Lichroprep). The column was eluted with methanol at a flow rate of 9–12 mL/min by using a Gilson Model HM detector set at 280 nm. A heart cut of the emerging peak was taken. The solvent was removed in vacuo and the product used in the next step. For removal of impurities with longer retention times from the column, 250 mL of 1:1 methanol–toluene was passed through the column. The limited column capacity precluded higher loading levels.

Silica Gel Chromatography. Fifty milligrams of product (I) from the reverse-phase chromatography step was dissolved in 4 mL of 4% ethyl acetate and 0.5% isopropyl alcohol in hexane and applied to a 2.2 × 20 cm column dry packed with 20 g of 230–400-mesh Lichrosorb silica gel 60. The sample was eluted with the same solvent system. A heart cut of the emerging peak measured on the Gilson detector was taken. The product was eluted with a total volume of 300 mL of eluant. The column was cleansed of impurities by eluting with a 300-mL solution of 50% ethyl

acetate and 0.5% isopropyl alcohol in hexane. The mass spectrum of the product corresponded to that of I (Deinzer et al., 1978): mp 212–214 °C [lit. mp 208 °C (Denivelle et al., 1959)]. Anal. Calcd for $C_{12}H_1Cl_9O_2$: C, 29.05; H, 0.20; Cl, 64.30. Found: C, 29.13; H, 0.26; Cl, 64.26.

RESULTS AND DISCUSSION

The spectral (IR 1688, 1577 cm^{-1}) and physical [mp 174–175 °C (lit. mp 177 °C)] properties of the nitric acid oxidation product of pentachlorophenol are consistent with those previously reported for 2,3,4,5,6-pentachloro-4-(pentachlorophenoxy)-2,5-cyclohexadienone (II) (Reed, 1958; Denivelle et al., 1959). The isomer 3,4,4,5,6-pentachloro-2-(pentachlorophenoxy)-2,5-cyclohexadienone, which Denivelle et al. (1959) obtained by treatment of II with ferric chloride at elevated temperatures, reportedly melted at 178 °C and had relevant infrared absorptions at ν 1695 and 1585 cm^{-1}. Isomer assignment of the latter compound was made on the basis that the reduction product, presumably I, was converted thermally to octachlorodibenzo-*p*-dioxin. Our own efforts to obtain additional structure confirmation for II by ^{13}C NMR analyses were unsuccessful, since the compound apparently was not stable under conditions of the NMR experiment, i.e., in CCl_4 with added Me_2SO and chromium acetylacetonate for nuclear relaxation. The instability of II has been alluded to before (Denivelle et al., 1959).

PCP reportedly is produced when II is treated with sodium iodide in acetone (Reed, 1958) or under acidic conditions (Denivelle et al., 1959). In methanol it apparently is reduced to nonachloro-4-phenoxyphenol by sodium iodide (Denivelle et al., 1959). Under our conditions in methanol and chloroform at room temperature, II is reduced to I in 31% yield, and the ratio of I to its isomer, nonachloro-4-phenoxyphenol, was 20:1. The melting point of I is 212–214 °C [lit. mp 208 °C (Denivelle et al., 1959)]. The isomer, nonachloro-4-phenoxyphenol, melts at 215–216 °C (Deinzer et al., 1980) [lit. mp 212 °C (Denivelle et al., 1959)]. Mass spectral data for I are consistent with those of the product isolated from PCP (Deinzer et al., 1978), and the quantitative conversion of a basic solution of I in the presence of 18-Crown-6 catalyst to octachlorodibenzo-*p*-dioxin (Deinzer et al., 1980) was demonstrated. For lack of sufficient evidence, we cannot offer an unambiguous mechanism for the rearrangement of II that leads to I during the reduction with sodium iodide solution. However, preliminary ESR studies give evidence for free radical intermediates, indicating that electron transfer from iodide to II occurs, giving an intermediate radical anion which may dissociate to give a pentachlorophenoxy radical and anion pair. These intermediates might recombine to give an ortho-substituted product, before being reduced further to the anion of I. When II is reduced with sodium borohydride, nonachloro-4-phenoxyphenol is obtained (Deinzer et al., 1980).

Impurities were removed from I by a combination of chromatographic methods. Ion-exchange chromatography was used to remove most of the residual pentachlorophenol, and extraction of the basic solution of I with trimethylpentane removed neutral compounds, leaving a product with a purity estimated to be ≥99%. The chromatographic cleanup methods for removal of trace impurities are based on the flash chromatography method of Still et al. (1978). The reverse-phase RP-18 column was used primarily to remove residual dioxins and other neutral compounds present with greater retention volumes than the chlorinated phenoxyphenol. Subsequent cleanup on a polar silica gel column removed polar impurities such as residual pentachlorophenol and nonachloro-4-phenoxyphenol.

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