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of a dark, DMF-insoluble residue. The purity was 75–80%.

The procedure described in the Experimental Section provided purified hesperidin in yields of 80–85% based upon the contents of the original mixture. The product was obtained as a white fluffy solid that was shown by standard methods to be quite pure. Analysis by gradient-elution HPLC (Figure 1) detected the presence of a minor, less polar impurity. This impurity was identified (vide infra) as isosakuranetin 7-β-rutinoside (2).

The acidic degradation of hesperidin into hesperetin (3), rhamnose, and glucose was first performed nearly 100 years ago (Tiemann and Will, 1881). The original method, which employs ethanolic aqueous sulfuric acid at elevated temperatures, has been utilized by most modern workers (Haley and Bassin, 1951; Sastry and Row, 1960; Looker and Holm, 1960) with little modification. It suffers in that hesperidin is poorly soluble in the medium and therefore extended reaction times or pressure vessels are required. Furthermore, the high temperatures and extended reaction times cause sugar resinification leading to the isolation of a discolored and impure product. Our investigations showed that recrystallization will not effectively remove these dark contaminants. Absorbents, such as activated carbon or acidic alumina, will clean up the product, but not without drastically lowering the yield.

A modification (Wilson, 1955) of the hydrolysis that significantly reduces the reaction time involves first dissolving hesperidin in dilute alkali and then adding this solution to refluxing aqueous hydrochloric acid. This procedure has been further improved by the use of 2-methoxyethanol as cosolvent (Dawson and Otteson, 1975). Hesperetin is still, however, provided containing yellow-brown resinous contaminants.

Of the range of methods investigated for performing this conversion, the utilization of neat methanol as the reaction solvent (a modification of the conditions of Arakawa and Nakazaki, 1960) was found to be vastly superior. Because hesperidin is quite soluble in this medium at reflux, the cleavage could be carried out in less than 8 h for a 5% solution in methanol–96% sulfuric acid (95:5). Little discoloration was afforded because of the lower boiling point of the solvent and the rapid rate of the reaction. Precipitation of an acetone solution of the methanolysis mixture into boiling water provided hesperetin in 99% yield as a slightly off-white, crystalline solid.

Gradient-elution, reverse-phase HPLC (Figure 1) showed the presence of a minor (~5%), less-polar impurity in the hesperetin. Coinjection with a series of citrus flavonoid aglycons (see Experimental Section) identified the impurity as isosakuranetin (4). The impurity present in the purified hesperidin was then readily identified as isosakuranetin 7-β-rutinoside (2) by coinjection of the hesperidin with 2 and poncirin (isosakuranetin 7-β-neo-hesperidoside), both potential precursors to the impurity found in the hesperetin.

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Toxaphene Degradation in Estuarine Sediments

Toxaphene in anoxic salt marsh sediments was degraded within a few days to compounds having gas chromatographic retention times shorter than those of standard toxaphene components. This breakdown occurred in sterile as well as unsterile sediments and also in a sand–Fe(II)/Fe(III) system. No breakdown was noticed in a sand system that did not contain the iron redox couple.

Toxaphene is presently the most heavily used chlorinated insecticide in the United States. Domestic consumption averages 26 million kg/year, with a cumulative total of over 500 million kg during the last 30 years (Guyer et al., 1971; vonRumker et al., 1974). The pesticide is made by photochemically chlorinating camphene in the presence

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of catalysts and is a complex mixture of at least 177 chlorinated components containing 67–69% Cl (Holmstead et al., 1974).

The average lifetime of toxaphene in agricultural soils is about 5 years, with volatilization probably the most important loss mechanism (Guyer et al., 1971; Nash et al., 1977). Once in the atmosphere toxaphene can be carried long distance from the source and has been found in ng/m^3 concentrations in air samples collected hundreds of kilometers out to sea (Bidleman and Olney, 1975).

During the spring and summer 1976, we measured toxaphene concentrations in rain along the South Carolina coast ranging from 13–195 ng/L (mean 75 ng/L, eight samples). Since toxaphene inhibits bone development in young fish at concentrations as low as 50 ng/L (Mehrle and Mayer, 1975a,b), it is important to understand the input and fate of toxaphene in estuaries. Aside from studies of toxaphene movement in an industrially contaminated estuary (Durant and Reimold, 1972; Reimold and Durant, 1974), no investigations of the fate of this pesticide in a salt marsh environment have been reported. Salt marsh sediments became anaerobic within a few centimeters of the surface (Fenchel and Riedl, 1970; Holland and Dean, 1977). Murthy et al. (1977) recently reported that toxaphene was reductively dechlorinated in anaerobic silt loam, and Khalifa et al. (1976) found that toxaphene was quickly degraded to compounds having shorter GC retention times by an iron(II) protoporphyrin system. Here we report the degradation of toxaphene in anoxic salt marsh sediments.

MATERIALS AND METHODS

Sediment was taken from the Baruch Plantation, an estuary near Georgetown, S.C., which is uncontaminated by industries or human habitation and which receives little fresh water input other than through rainfall. The samples were collected at low tide from within the reducing layer of the sediment, about 20 cm depth. The pH of the sediment was 6.5, and the redox potential (-150 mV, measured with a Pt electrode vs. SCE) was within the range of potentials measured in water-logged soils by Glass (1972) in his studies of DDT reductive dechlorination. Analysis of the top 5 cm of the sediment column revealed no detectable toxaphene, although low levels of other chlorinated pesticides and PCB (Aroclor 1254) were found (ng/g of dry weight): p,p'-DDE = 1.8, p,p'-DDD = 1.9, PCB = 3.0.

Toxaphene degradation was studied in four systems: (a) sterilized sediment (autoclaved for 1 h on 2 successive days), (b) unsterilized sediment, (c) sterilized sand containing $Fe_3(OH)_8$, the Fe(II)/Fe(III) redox couple described by Glass (1972); pH = 5.6, -250 mV vs. SCE, and (d) sterilized sand plus distilled water ("control"). Toxaphene was added to all four systems at 5 $\mu g/g$ (5 ppm) of wet sand or sediment, and the systems were kept in glass–stoppered flasks at room temperature under nitrogen.

The contents of the flasks were sampled every 2 days and the chlorinated hydrocarbons were isolated from 10 g of sand or sediment by mechanical agitation (sand systems) or Soxhlet extraction (sediment systems) with 150 mL of CH_3CN. Distilled water (400 mL) was added to the CH_3CN and the chlorinated hydrocarbons were partitioned into two 100-mL volumes petroleum ether. The extracts were cleaned up by alumina column chromatography (Woelm activity grade III, 4 g), shaken with 7% fuming H_2SO_4, followed by metallic Hg to remove oxygen- and sulfur-containing compounds, respectively, and analyzed by ^{63}Ni electron-capture gas chromatography. Analyses were carried out in a Tracor Microtek 222 chromatograph,
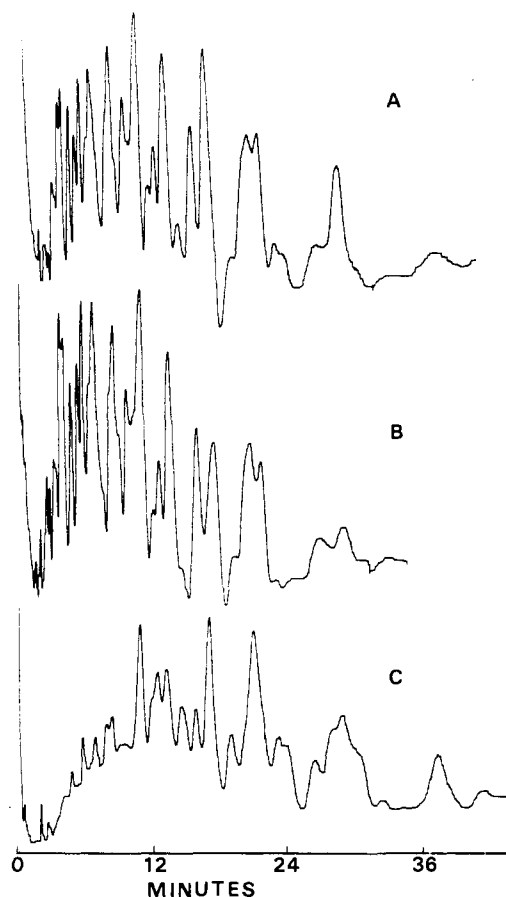
Figure 1. Gas chromatograms of toxaphene degraded in sterilized salt marsh sediment: (A) 2 days after toxaphene addition, (B) 6 days after toxaphene addition, (C) undegraded toxaphene standard.

using a 180-cm 1.5% OV-17/1.95% OV-210 column operated at 200 °C and 60 mL/min N_2 flow.

All chemicals were reagent grade and all solvents were Mallinckrodt Nanograde brand. Technical grade toxaphene containing 67–69% Cl and yielding a GC fingerprint identical with that of the Environmental Protection Agency standard reference material was used to spike the degradation systems.

RESULTS AND DISCUSSION

Toxaphene was quickly degraded in both the sterile and unsterile sediments and in the sand containing the Fe-(II)/Fe(III) redox couple. As in Khalifa et al.'s (1976) study of toxaphene breakdown, we were unable to quantitate the extent of degradation with the electron-capture detector. Nevertheless, it was evident that the normal toxaphene fingerprint was greatly altered within a few days by the formation of compounds having shorter GC retention times (Figure 1). That these changes in the sediment system were caused by chemical rather than biological processes was suggested by the breakdown of toxaphene in sterile as well as unsterile samples and was confirmed by the similar GC pattern of products isolated from the Fe(II)/Fe(III)–sand system (Figure 2). No breakdown of toxaphene in the sand control system (d) was noticed within the time period of our experiments (14 days). The breakdown of toxaphene in salt marsh sediments and in the Fe(II)/Fe(III)–sand system probably occurs by a reductive dechlorination mechanism, as discussed by Glass (1972) for the conversion of DDT to DDD.

Rain collected in the Baruch Plantation in 1976 contained toxaphene levels 10–100 times higher than those
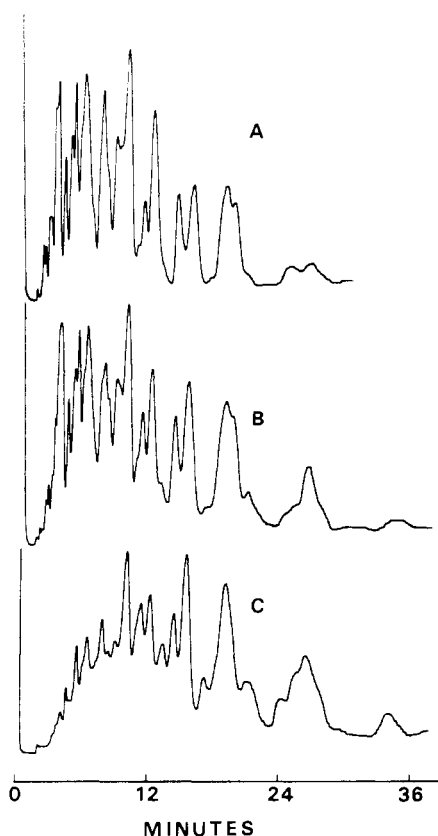
Figure 2. Gas chromatograms of toxaphene degraded in (A) sterilized salt marsh sediment (8 days after addition) and (B) sand–Fe(II)/Fe(III) system (14 days after addition). Chromatogram C is an undegraded toxaphene standard.

of other chlorinated pesticides (DDT, DDE), yet although we found parent toxaphene components in oysters, we could not identify toxaphene in the sediments. At the time of these analyses, however, we would not have recognized chromatograms such as Figure 1a as indicative of toxaphene degradation products and may have overlooked them. Thus, while toxaphene in anoxic marsh sediments appears to undergo changes in composition very quickly, the ultimate breakdown and toxicity of the alteration products has yet to be determined.

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Colorimetric Determination of Browning Precursors in Orange Juice Products

A colorimetric method for the determination of browning precursors in orange juice products is proposed, based on the thiobarbituric acid (TBA) color reaction with 5-hydroxymethylfurfural (HMF). Two clarification methods (Carrez solutions and saturated lead acetate) and several reaction conditions were evaluated for color complex intensity, stability, and repeatability. Pulp precipitation by lead acetate is recommended for the clarification step. A color complex, stable for up to 60 min, was formed in a reaction mixture of 2 mL of clear serum, 2 mL of trichloroacetic acid 40% w/v solution, and 1 mL of 0.05 M TBA, treated for 50 min at 40 °C. The proposed method offers some advantages in repeatability and convenience of HMF determination, in comparison to previously reported colorimetric or TLC procedures. Formation of HMF during storage in freeze-dried orange crystals, qualitatively evidenced by TLC and GLC methods, was measured by the suggested procedure.

Orange and other citrus juice products are susceptible to various deterioration reactions during processing and storage, resulting in off-flavor development and browning. The chemical changes have been extensively investigated during the last three decades, and the main deterioration mechanisms considered to cause off-flavor buildup include degradation of essential oil and aroma compounds (Blair et al., 1952; Bielig et al., 1972; Askar et al., 1973a,b),