most exactly the same as that produced by sodium hydroxide dehydrochlorination, and the total recoveries suggest that no other types of reactions were occurring.

Influence of Extraction Procedure. Fat, extracted by the four different procedures referred to under Experimental Section, was examined for its ability to subsequently induce the γ-HCH and pp'-DDT degradations over 4 days and the products were examined after both the Telling and AOAC cleanup procedures (Table V).

It appears, therefore, that the degradation is induced by some heat-labile factor extractable from egg substrates. The losses were almost entirely accounted for by identification and quantification of the byproducts listed above, although the AOAC method leads to lower figures overall.

When "active" egg fat was heated by gentle refluxing in hexane for 4 h and then tested for its ability to decompose α-HCH at room temperature during 6 days, about two-thirds of the facility to induce degradation had been lost. Washing the active egg fat with dilute acid (0.1 N) completely destroyed its ability to degrade α-HCH and no active constituent could be recovered by neutralizing the acid wash.

The heat-labile factor responsible for the degradations could well be a natural component of hen's eggs. Preliminary tests showed that, although some amines may be capable of dehydrochlorinating α-HCH (and not pp'-DDE) in nonpolar solvents, potential mediators from eggs such as phosphatidylethanolamines or trimethylamine, derived from poultry feed, were unlikely to be responsible. The observed effect is unlikely to arise from an artifact in the analytical method or be of microbial origin.

It is concluded that, when analyzing eggs for organochlorine pesticide residues, the initial fat extract should not be stored, particularly if obtained by using solvents at room temperature, but immediately cleaned-up for the pesticide(s) to be determined by GLC. Analysis of freeze-dried material may likewise not correspond with the original levels of certain organochlorine pesticides in the fresh eggs because of dehydrochlorination-type reactions in situ.

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Importance of Nootkatone to the Aroma of Grapefruit Oil and the Flavor of Grapefruit Juice

The influence of nootkatone on the aroma of cold-pressed grapefruit oil and the flavor of grapefruit juice flavored with either the oil, limonene, or nootkatone in limonene was studied. Nootkatone had more effect on the aroma of the oil than on the flavor of juice flavored with the same oil. The aroma of oil with a naturally high nootkatone level was usually distinguishable from that of other samples. The frozen concentrated juice used in this study contained nootkatone at slightly above its threshold level prior to addition of oil despite its apparent low oil content. The aroma and taste panel results suggest that several other components of grapefruit oil are essential to good grapefruit aroma and flavor in addition to nootkatone.

Since MacLeod and Buigues (1964) first reported nootkatone as a flavor impact compound in grapefruit, nootkatone content has been suggested as a quality index standard in grapefruit oil (MacLeod, 1966), and synthetic nootkatone has been used in some grapefruit-flavored beverages (Shaw, 1978). As a result of their personal experiences with synthetic nootkatone, several food flavorists have raised the question to us as to what extent nootkatone influences grapefruit flavor and whether it is necessary for good grapefruit flavor.

MacLeod and Buigues (1964) stated that in sugar solutions the flavor threshold of nootkatone was 20–40 ppm and that the odor was detectable below 10 ppm. Berry et al. (1967) found a flavor threshold for nootkatone of 1 ppm in water and 6 ppm in grapefruit juice. Haring et al. (1972) reported an odor threshold of 0.8 ppm in water and 30 ppm

in air for (+)-nootkatone, the enantiomer present in grapefruit. Stevens et al. (1970) found an odor threshold in water of 0.15 ppm for crystalline nootkatone isolated from grapefruit oil. The mother liquor from the crystallization was far more potent, and its aroma was judged more grapefruit-like than that of crystalline nootkatone, thus indicating the presence of other important flavor components in grapefruit oil.

In the current study, we report the relationship of nootkatone to the aroma of grapefruit oil and the flavor of grapefruit juice, as determined by an experienced taste panel. The panel judged the level of nootkatone in grapefruit oil to be more important to the aroma of the oil than to the flavor of grapefruit juice containing the same oil.

EXPERIMENTAL SECTION

Commercial early- and late-season cold-pressed grapefruit oils known to contain relatively low and high nootkatone levels, respectively, were obtained from Citrus Central, Inc., Orlando, FL. A commercial sample of frozen concentrated grapefruit juice (59 °Brix; 0.002% residual oil) containing no added oil or other flavor fractions was used to prepare the juice samples for flavor testing. Oil (117 μL) was added in portions with a syringe to 177 mL of concentrated juice with slow stirring prior to reconstitution to single-strength juice (10.5 °Brix; 0.012% oil). (+)-Limonene, P&F grade (Glidden Organics, Jacksonville, FL) was 99.6% pure by GC, and (+)-nootkatone, mp 34–35 °C, was 99.5% pure by GC.

Quantitation of Nootkatone in Oils and in Single-Strength Grapefruit Juice. Nootkatone content of the cold-pressed grapefruit oils (Table I) was determined by the glass capillary gas chromatographic method reported earlier (Wilson and Shaw, 1980), except that a 30-m fused silica SP-2100 column was used.

Portions (50 μL each) of single-strength grapefruit juice reconstituted from commercial 59 °Brix frozen concentrated grapefruit juice containing no added oil or other flavor fractions were spotted within 1 cm wide bands on a silica gel G plate along with nootkatone standards prepared in ethanol but representing 2, 4, 6, 8, and 10 ppm of nootkatone/50 μL of juice. Plates were developed in one pass and sprayed as described earlier (Tatum and Berry, 1973). Either 195:5 benzene–acetone or 135:65:0.5 chloroform–ether–acetic acid was used as solvent system; and dried plates were sprayed with 10% sulfuric acid in ethanol, heated at 125 °C for 5 min, and then viewed under ultraviolet (UV) light so that nootkatone could be identified and its quantity estimated (Tatum and Berry, 1973). Nootkatone appeared as a yellow fluorescent spot under UV light. After development with either solvent system, the single-strength grapefruit juice was judged by visual comparison with the standards to contain ~7 ppm of nootkatone (Tatum and Berry, 1975).

Aroma and Flavor Panel Evaluations. Oils were judged by a paired comparison difference test, with 12 experienced taste panel members, using two presentations at least 20 min apart for each panel member to minimize fatigue (Boggs and Hanson, 1949). Single-strength grapefruit juice samples containing appropriate levels of the above oil samples were judged by a triangle comparison difference test, with two presentations each to 12 experienced taste panel members (Boggs and Hanson, 1949).

RESULTS AND DISCUSSION

The relationship of nootkatone to the aroma of cold-pressed grapefruit oil and the flavor of grapefruit juice was studied by comparison of two oils containing relatively high (0.83%) and relatively low (0.02%) levels of nootkatone.

Table I. Effect of Nootkatone (noot.) Content on the Aroma of Grapefruit Oil and the Flavor of Grapefruit Juice

oil samples compared[a]	confidence limit, % aroma panel with oils[b]	confidence limit, % flavor panel with juices[c]
limonene vs. high-noot. oil		99.9
limonene vs. noot. in limonene	99	NS[d]
noot. in limonene vs. high-noot. oil	99	95
low-noot. oil vs. high-noot. oil	NS	NS
noot. plus low-noot. oil vs. high-noot. oil	95	NS

[a] High-nootkatone oil, nootkatone in limonene, and nootkatone plus low-nootkatone oil all contain 0.83% nootkatone; low-nootkatone oil contains 0.02% nootkatone. [b] Paired comparison difference test. [c] Triangle comparison difference test. [d] NS = not significant at a confidence level of 95% or greater.

These levels are near the two extremes for nootkatone content in acceptable-quality grapefruit oils (Kesterson et al., 1971). Table I lists the results of aroma tests on the oils (neat) and flavor tests on the oils added to single-strength grapefruit juice prepared from "evaporator pumpout" (frozen concentrated juice to which no flavor fractions had been added back). Generally, aroma panels were conducted first, and the oils were then added to frozen concentrated grapefruit juice as soon as possible for flavor tests on reconstituted juice. An initial flavor test was run with limonene vs. high-nootkatone oil (Table I) to make sure the panel could easily distinguish the flavor of grapefruit oil in the juice sample used for all subsequent tests. In cases where no grapefruit oil was added to the evaporator pumpout, an equivalent quantity of limonene was added to provide the oily flavor note present in good-quality citrus juices.

Two tests were conducted to see if nootkatone in limonene could be distinguished from other samples. Limonene and nootkatone in limonene were distinguishable in the aroma tests (when only the oils were considered), but when these samples were added to juice, the panel was unable to distinguish the sample containing nootkatone. With nootkatone in limonene vs. grapefruit oil containing the same level of nootkatone, the panel was able to distinguish both the aroma and flavor samples that contained the grapefruit oil. These results indicate the importance of oil components other than nootkatone to the flavor imparted by grapefruit oil added to juice.

Two tests were conducted to study the overall influence on grapefruit flavor of oils containing varying levels of nootkatone. In the first test, low-nootkatone oil was indistinguishable from high-nootkatone oil either by aroma of the neat oils or by flavor of juice containing the oils at identical levels (Table I). However, when crystalline nootkatone was dissolved in low-nootkatone oil to raise the nootkatone level to that of the high-nootkatone oil, the panel could distinguish the aromas of the two oils at the 95% confidence level, although not the flavor of juices containing the two oils. Apparently, the added nootkatone modified the aroma of the low-nootkatone oil, perhaps through synergistic effects with other components of the oil. Comparison of the gas chromatographic profiles for the two oils showed seven unidentified peaks, with elution times between 18 and 28 min, present in the nootkatone-fortified oil but not in the unfortified high-nootkatone oil. Several panel members noted an off-flavor (overripe or

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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