and 120 which matched with the mass spectrum of the L-phenylalanine methyl ester standard.

Figure 3 shows the decomposition pattern of APM stored at the same conditions as the APM hydrochloride. The pH of the 1% solution of APM was 4.6. Though APM was degraded to a much lower degree than APM hydrochloride, the presence of the same degradation products was detected.

The glc analysis of APM and its degradation products may be a useful tool for the investigation of APM stability in different systems.

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Germacrene D in Douglas Fir Young Needles

A sesquiterpene hydrocarbon which exhibits a transitory existence in the young needles of Douglas fir, *Pseudotsuga menziesii* (Mirb.) Franco, has been identified by spectroscopic and gas chromatographic techniques as germacrene D.

In an earlier investigation of the changes in composition of volatile terpenes in Douglas fir needles during maturation (Maarse and Kepner, 1970) we observed the transitory existence of a sesquiterpene hydrocarbon (peak 25) in relatively large amounts in the young needles. This sesquiterpene reached a maximum concentration approximately 10 days after emergence of the new growth in the spring and then rapidly decreased in concentration as the needles matured. We also isolated the same component from the new needle growth of Douglas fir seedlings grown in the Aboretum of Schovenhorst at Putten, The Netherlands. This communication presents evidence identifying this sesquiterpene hydrocarbon as germacrene D.

EXPERIMENTAL SECTION

Material. Approximately 2 kg of Douglas fir new growth (tips about 2 cm in length) was collected from young Douglas fir trees located in the Pacific Coast Range west of Ukiah, Calif. The new tips were macerated in a blender and steam distilled at atmospheric pressure, the distillate extracted with *n*-pentane, and the pentane removed on a rotary evaporator at 0° to give 6.5 ml of oil. The oil was stored in a brown bottle at 0° under nitrogen until used for glc separations.

Gas Chromatography. Isolation of the sesquiterpene hydrocarbon was by preparative glc on a 10 ft × 0.16 in. i.d. glass column packed with 5% Carbowax 20M on 60–70 mesh Chromosorb G DMCS in a Hewlett Packard 7620A chromatograph with FID and a 15:1 exit splitter; oven temperature isothermal at 130° for 15 min, programmed at 2°/min to 160° then at 30°/min to 200°; injection port and detector 200°; He flow 60 ml/min. Final purification was by rechromatography on the above column and on a 10 ft × 0.16 in. i.d. glass column packed with 5% SE 30 + Igepal C0880 (5% w/w) on 60–70 mesh Chromosorb G DMCS. Analytical separations and Kovats Index determinations were on 500 ft × 0.03 in. i.d. stainless steel columns coated with Carbowax 20M + Igepal C0880 (5% w/w) or SF 96(50) + Igepal C0880 (5% w/w) in an F&M Model 810 gas chromatograph with FID.

Instrumental Analyses. Infrared analyses were taken between sodium chloride plates in a Beckman IR8 spectrophotometer using a beam condensor. Mass spectra were determined on a Consolidated Electrodynamics Corporation Model 201 spectrograph or on a Varian Mat CH4 mass spectrometer. Ultraviolet analyses were carried out on a Cary 14 Ultraviolet and Visible spectrometer in spectroquality *n*-hexane. Irradiation of the sesquiterpene hydrocarbon was carried out for 2 hr in a quartz nmr tube using spectroquality *n*-hexane as solvent with a Hanovia 350-W medium pressure mercury-arc lamp.

RESULTS AND DISCUSSION

The germacrene D (peak 25; Maarse and Kepner, 1970) isolated in this study gave infrared and mass spectra and Kovats' indices completely consistent with the data of Maarse and van Os (1973) and with the infrared and ultraviolet spectral data of Yoshihara *et al.* (1969) for germacrene D. Photoisomerization of germacrene D from Douglas fir gave mainly β-bourbonene (Yoshihara *et al.*, 1969), identified by its infrared spectrum (Wenninger *et al.*, 1967) and Kovats' indices (Maarse and van Os, 1973), and trace amounts of β-copaene and β-ylangene (Kovats' indices 1626 and 1609 on Carbowax 20M, and 1445 and 1433 on SF 96(50), respectively).

Yoshihara *et al.* (1969) reported the hydrogenation of germacrene D to give germacrane, identified by the infrared spectrum. Catalytic hydrogenation of the germacrene D from Douglas fir oil using platinum oxide (Adam's catalyst) at atmospheric pressure and 0° gave a complex mixture of products which separated into 20 peaks on SF 96(50). No evidence could be observed from mass spectra determination for any of the germacrane isomers in the products. Hydrogenation of germacrene D from Douglas fir oil in the gas chromatograph injection port over Pd at 190° (Kepner and Maarse, 1972; Maarse, 1974) gave a mixture of products (eight peaks on an SF 96(50) capillary column) identical with the products obtained by similar hydrogenation of germacrene D isolated from Origanum oil. Mass spectral evidence (Maarse, 1974)

indicated three germacrane isomers present in this mixture in addition to cyclization products (eudesmanes and a cadinane).

When the Douglas fir oil was stored at −20° in a freezer there was no decrease in the amount of germacrene D present after 1 year, but when the oil was stored at 5° in a refrigerator the peak disappeared in 6 weeks. Yoshihara *et al.* (1969) reported that thermal isomerization of germacrene D gave principally the same products as acid-catalyzed isomerization. Carefully purified germacrene D from Douglas fir, sealed neat in glass capillaries or dilute in polar or nonpolar solvents, showed no sign of thermal rearrangement to other sesquiterpenes after 6 weeks at room temperature in the dark. The loss of germacrene D in the Douglas fir oil stored at refrigerator temperature would thus appear not to be a simple thermal isomerization but must involve interaction with some component of the oil. The possibility that such interaction might involve a terpene alcohol was explored, but a sample of germacrene D sealed in hexane with 5% added linalool showed no isomerization to other sesquiterpenes after several weeks at room temperature. In all attempts to isomerize germacrene D thermally and in the whole oil stored at refrigerator temperature, small amounts of a white crystalline high-melting solid were observed which was apparently a polymeric product of the germacrene D.

Germacrene D has recently been reported as present, usually in relatively small amounts, in a variety of plant species (Lawrence *et al.*, 1971, 1972, 1974; Maarse and van Os, 1973; Nishimura *et al.*, 1969; Yoshihara *et al.*, 1969). In Douglas fir needles the amount of germacrene D reaches a maximum concentration (*ca.* 5% of the isolated essential oil) in the very young growth and then decreases to essentially zero as the growth matures. Maarse (1975) observed similar variations in the amounts of germacrene D in leaves of *Origanum vulgare* with age of the leaves. von Rudloff (1972, 1973) investigated the leaf oil of many different populations (coastal and interior) of Douglas fir but found no germacrene D in mature leaves or foliage. This is consistent with our observations of the transient existence of germacrene D in the very young leaves. Germacrene D has been suggested (Yoshihara *et al.*, 1969) to be a key intermediate in the biogenesis of a number of sesquiterpene hydrocarbons. Although the germacrene D decreases in amount rapidly as the leaves of Douglas fir (Maarse and Kepner, 1970) and *Origanum vulgare* (Maarse, 1975) mature, in neither case can an increase in concentration of sesquiterpenes chemically or biogenetically related to germacrene D be observed. It is thus not possible to conclude from the results obtained whether or not germacrene D is a precursor for other sesquiterpenes present in these plants.

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Determination of Amines in Fresh and Processed Pork

The concentration of a number of amines was determined in fresh, cooked, smoke-cured, and putrefied pork. Analyses were conducted for spermine, spermidine, putrescine, cadaverine, histamine, tyramine, tryptamine, and ethanolamine. The amines were recovered from perchloric acid extracts of the lean meat and derivatized with 1-dimethylaminonaphthalene-5-sulfochloride. The fluorescent derivatives were separated by thin-layer chromatography, extracted, and then quantitated spectrofluorometrically. The concentration per 100 g of fresh tissue ranged from 0.5 mg for tyramine to 189 mg for putrescine. Significant increases in spermine, spermidine, putrescine, and cadaverine occur during putrefaction. Cooking at 71° decreases the concentration of amines.

N-Nitrosamines have been found to be carcinogenic to animals (Magee and Barnes, 1956). These compounds are formed by the reaction of nitrite with secondary amines. Since cured meat products are prepared with nitrite, there is considerable interest in the components of meat, such as amines, that could react with nitrite to form nitrosamines. Crosby *et al.* (1972) detected traces of the following nitrosamines in food: dimethylamine, diethylamine, pyrrolidine, and piperidine. Lijinsky and Epstein (1970) postulated that cadaverine and putrescine upon heating formed piperidine and pyrrolidine, and Bills *et al.* (1973) demonstrated the production of *N*-nitrosopyrrolidine from such polyamines as spermidine and putrescine when heated in the presence of sodium nitrite.

Information concerning the concentrations of free amines in fresh and processed foods has been scant until