Table III. Simple Correlations between Connective-Tissue Components and Chronological Age of 20 Pork Loins

	Fraction C, Dilute Acid- and Alkali-Insoluble Collagen	Fraction D, Elastin	Fraction A, Acid-Soluble Collagen	Fraction B, Alkali-Soluble Collagen	Fractions C and D	Fractions A and B	Fractions B/A
Fraction D	0.92[a]						
Fraction A	−0.03	−0.22					
Fraction B	−0.65[a]	−0.65[a]	0.23				
Fractions C and D			−0.15	−0.66[a]			
Fractions A and B					−0.60[a]		
Age[b]	−0.31	0.07	−0.48[c,d]	0.37	−0.08	0.19	0.59[c]

[a] $P = 0.01$. [b] $n = 11$. [c] $P = 0.05$. [d] Partial correlation coefficient; % intramuscular fat statistically held constant.

Acknowledgment

The authors thank the Oscar Mayer Co., Madison, Wis., for assistance with the palatability and processing phases of the study.

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Received for review February 21, 1964. Accepted May 15, 1964. Research published with the approval of the Director of the Wisconsin Agricultural Experiment Station, Madison, Wis. Supported in part by research contract 12-25-010-577, U. S. Department of Agriculture, Market Quality Research Division.

ESSENTIAL OILS

Sources of Monoterpene Hydrocarbons

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Oils of savin, cubeb, sweet marjoram, galbanum, thuja, bay, lemon, and orange were investigated as sources for authentic samples of α-thujene, 3-carene, sabinene, α-terpinene, γ-terpinene, myrcene, β-phellandrene, and terpinolene. Also analyzed were secondary components of commercial samples of terpenes. α-Terpinene, γ-terpinene, and terpinolene were obtained by silica gel isomerization of limonene. Infrared Spectra of α-thujene, 3-carene, sabinene, γ-terpinene, myrcene, and terpinolene are presented.

OBTAINING authentic samples of specific monoterpene hydrocarbons is frequently difficult. Some of the more common terpenes, such as α-pinene, β-pinene, limonene, α-phellandrene, camphene, and *p*-cymene, can be obtained from chemical supply houses, but their purity, and occasionally their authenticity, are questionable. A variety of essential oils have been reported to contain certain of the monoterpenes, but identification has sometimes been limited to the evidence of gas chromatographic retentions. Because of a need for a more complete stock of these compounds for use as chromatographic standards, the authors were interested in establishing sources for these materials. Among the compounds of particular interest were sabinene, α-thujene, 3-carene, α-terpinene, γ-terpinene, myrcene, β-phellandrene, and terpinolene.

Although gas chromatography is a powerful tool for separating complex mixtures, relying on it as a sole source of identification is unwise. Once the composition of a mixture is established and evidence of specific compounds is conclusive, gas chromatography becomes somewhat more reliable for determining the incidence and estimating the quantity of a particular compound in a natural mixture.

These investigations involve gas chromatographic separation and infrared characterization of fractions isolated from oils of savin, cubeb, sweet marjoram, galbanum, thuja, bay, lemon, and orange. Also examined were fractions contained in commercial samples of terpenes (which are, unfortunately, rarely pure).

Apparatus

Preparative-scale gas chromatography utilized an Aerograph Autoprep with a 20-foot by 1/4-inch aluminum column

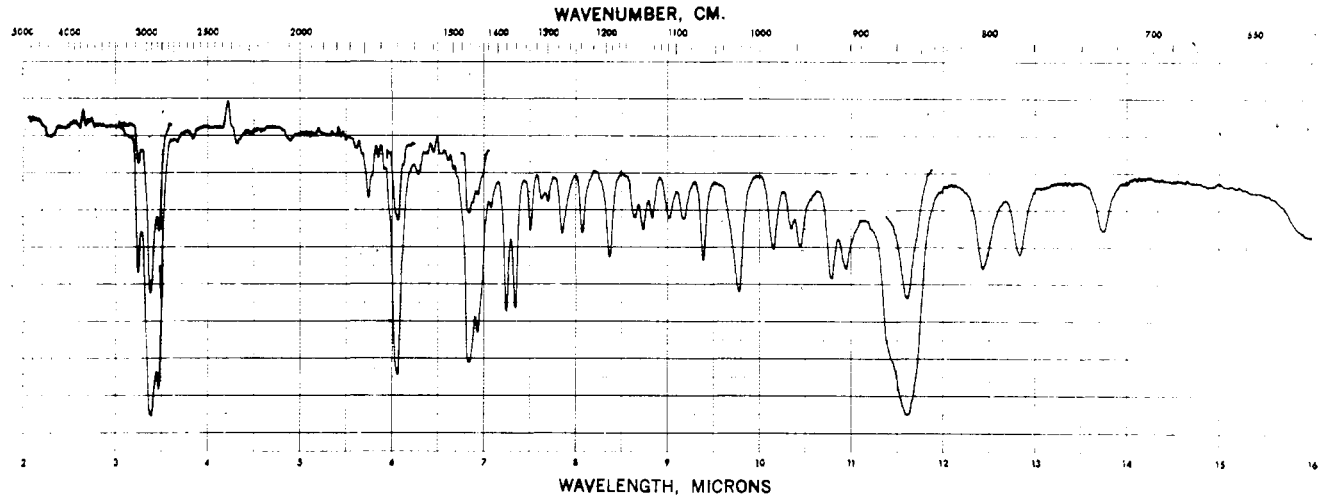

Figure 1. Infrared spectrum of sabinene

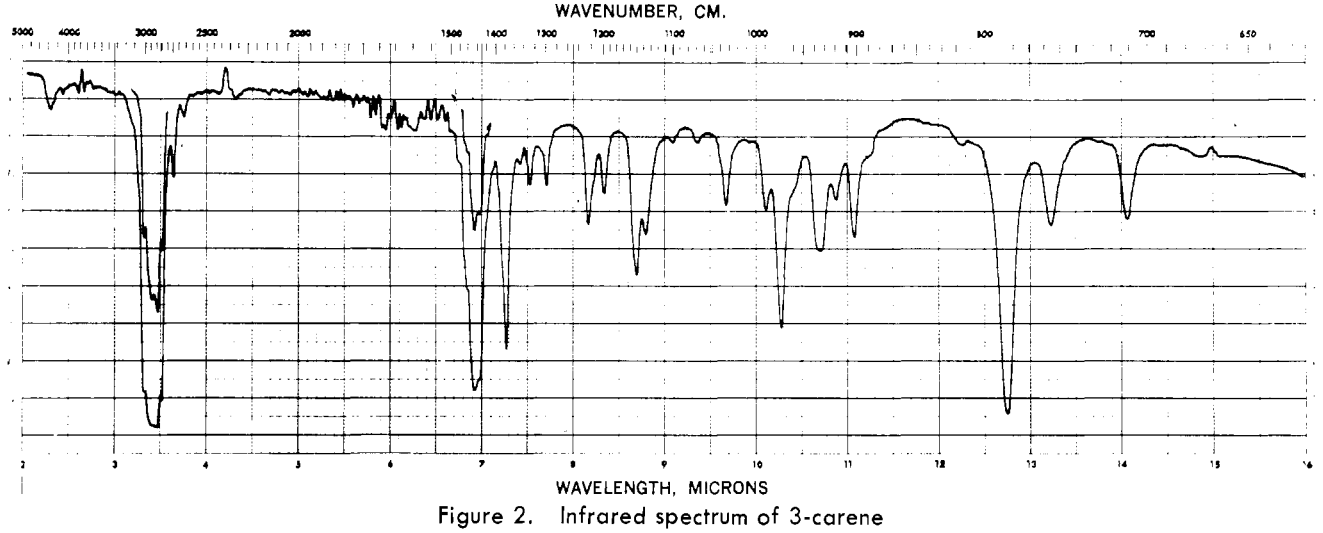

Figure 2. Infrared spectrum of 3-carene

packed with 40–60 HMDS-treated Chromosorb P coated with 15% Carbowax 20 M, and an Aerograph Model A-90, fitted with a 10-foot by $^1/_4$-inch stainless steel column similarly packed.

Analytical chromatograms utilized the Aerograph Hy-Fi, with a 10-foot by $^1/_8$-inch stainless steel packed column (identical substrate) and flame ionization detection.

Vacuum distillations utilized a semimicro-Podbielniak column with an efficiency of 75 theoretical plates.

Infrared spectra were determined on a Beckman Model IR-5 spectrophotometer fitted with a Beckman beam condenser. Spectra were determined in either CIC Type D microcavity cells or the Beckman demountable ultramicro liquid cell. Ultraviolet spectra were determined on a Beckman DB spectrophotometer.

Reagents

Oils of savin, cubeb, sweet marjoram, galbanum, thuja, and bay were purchased from chemical supply houses. Oils of lemon and orange were obtained from R. A. Bernhard of this department.

Procedures

The various oils were subjected to vacuum distillation to isolate the monoterpene hydrocarbons. So that the distillate composition would be limited solely to monoterpene hydrocarbons, the distillations were rarely carried to completion. Because some monoterpene hydrocarbons therefore remained in the pot residues, the percentage of total monoterpene hydrocarbons in the original oils could not be calculated.

Relative retention times and percentage composition of individual fractions making up these distillates were calculated from the chromatograms of the analytical instrument. Percentage composition was estimated by calculating the percentage of the total peak area. Isolated fractions were collected from the A-90 chromatograph by inserting 1-foot lengths of thin-walled, glass capillary tubing in the outlet port when the desired fraction was being eluted (*8*).

Infrared spectra were determined as thin films; ultraviolet spectra were determined in Spectrograde isooctane. After spectra were determined, the homogeneity of the isolated fractions was rechecked by gas chromatography on the analytical instrument.

The gas chromatographs were operated isothermally. The preparative instrument was operated at 120° C., and a flow rate of 60 ml. of He per minute. The analytical instrument was operated at 100° C.; N_2, 20 ml. per minute, H_2, 20 ml. per minute.

Results and Discussion

Sabinene. Guenther (*4*) reported sabinene to be the main constituent of oil of savin, amounting to 30%, in contrast to the 20% reported by Booth (*2*). Rudloff (*10*) found the commercial oil to contain 26% sabinene, and distillate from the leaves of *Savin juniper* to contain 30.5%. Ikeda *et al.* (*6*) reported that the monoterpene hydrocarbon fraction constituted 33.4% of the oil of savin. Of these hydrocarbons, 59.4% was sabinene.

The infrared spectrum of the main fraction obtained from oil of savin (Figure 1) agreed with the spectrum of sabinene reported by Stanley, Ikeda, and Cook (*13*) and with that found by Teranishi (*14*). This compound was 48% of the monoterpene fraction. The infrared spectrum and retention data for another component comprising 20% of the oil were in agreement with those of α-pinene.

3-Carene. The Indian oil of turpentine of *Pinus longifolia* was reported by Guenther (*4*) to consist of 38% 3-carene. The present researchers were unable to obtain samples of this oil.

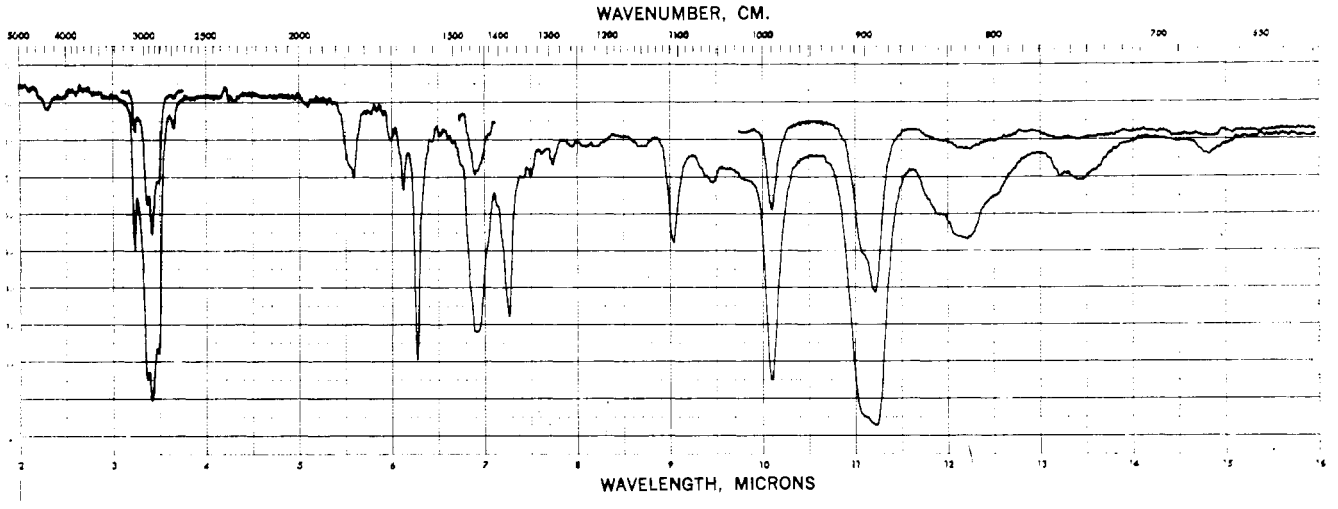

Figure 3. Infrared spectrum of myrcene

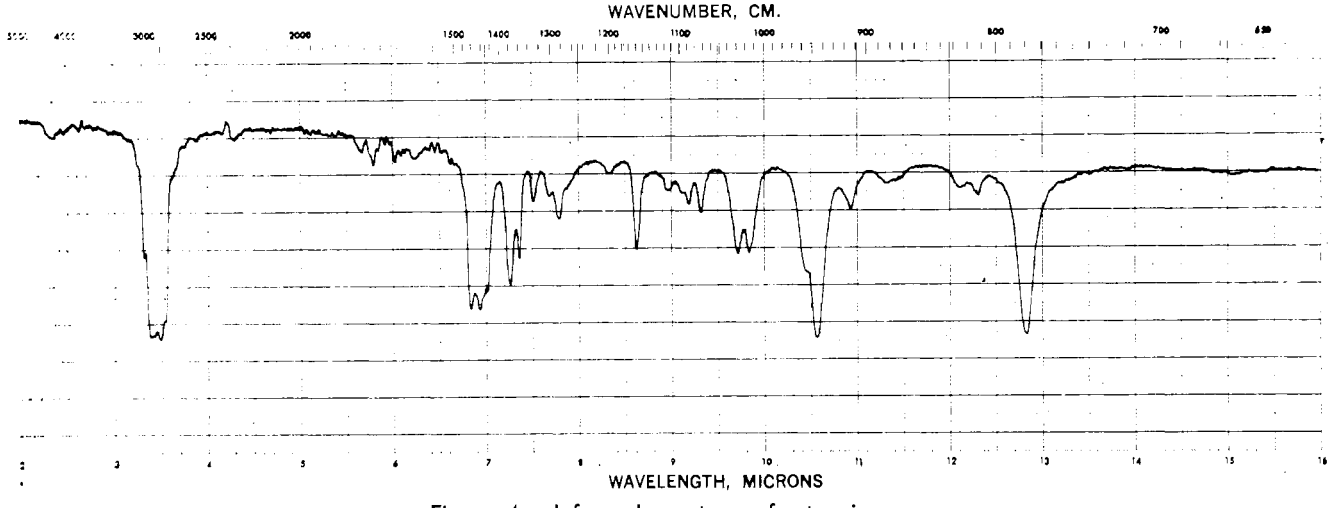

Figure 4. Infrared spectrum of γ-terpinene

Ikeda *et al.* (*6*) reported that the monoterpene hydrocarbon fraction of oil of galbanum consisted of 19.6% 3-carene. The current study indicated three main fractions in distillate from oil of galbanum. The infrared spectrum and retention data of fraction 1 (24%) and fraction 2 (52%) were identical to those of α- and β-pinene, respectively. The third fraction (21%) (Figure 2) agreed with 3-carene as reported by O'Connor and Goldblatt (*9*), except that the spectrum of the material isolated in this study, which did agree with that of Teranishi (*14*), exhibited a strong absorption at 12.75 microns. The difference can probably be attributed to the fact that O'Connor and Goldblatt determined their spectra in chloroform; hence absorption in this region would be masked.

Myrcene. Myrcene was reported as the main constituent of bay oil as early as 1895 (*4*). Ikeda *et al.* (*6*) reported that myrcene made up 70% of the monoterpene hydrocarbons of bay oil. A major fraction (69%) of the bay oil distillate utilized in this study absorbed at 225 mμ. Myrcene also exhibits this absorption, according to O'Connor (*9*), and the infrared spectrum published by those investigators is also in agreement with that of this fraction (Figure 3).

α- and γ-Terpinene. Guenther (*4*) reported that oil of sweet marjoram is 40% terpenes, mainly terpinene. Ikeda *et al.* (*6*) reported that the monoterpene hydrocarbon composition of this oil was 18.1% α-terpinene. In the authors' laboratory, however, the chromatograms of sweet marjoram distillate showed only less than 1% for the compound expected to be α-terpinene and 1% for the compound expected to be γ-terpinene on the basis of retention data on Carbowax 20 M published by Ikeda *et al.* (*6*).

γ-Terpinene has been reported in lemon oil—in small amounts by Guenther (*4*), 9.78% by Di Giacomo, Rispoli, and Crupi (*3*), and 10.3% by Stanley, Ikeda, and Cook, (*13*). In the authors' analysis of lemon oil the fractions expected to be α- and γ-terpinene were, respectively, present in quantities of less than 1 and 7%.

Italian sweet orange oil was listed by Guenther (*4*) as containing 8% α-terpinene. Teranishi detected γ-terpinene in aromagrams of orange juice (*15*), and Bernhard (*1*) reported γ-terpenine in orange oil. Schweisheimer (*12*) reported that the volatiles of orange skins were 90% *d*-limonene. The sample of that oil used in this study was 1% γ-terpinene and 74% limonene; no evidence of α-terpinene was obtained.

Hunter and Brogden (*5*) found that, in the presence of silica gel, limonene isomerized in 20 minutes at 100° C., yielding approximately 18% α-terpinene, 11% γ-terpinene, and 20% terpinolene. When their experiment was repeated in the authors' laboratory, limonene underwent isomeric rearrangements, yielding three main compounds thought to be α-terpinene (10%), γ-terpinene (5%), and terpinolene (11%). The infrared and ultraviolet spectra of the fraction thought to be γ-terpinene agreed with those reported by O'Connor and Goldblatt (*9*), and the infrared spectrum (Figure 4) was identical to Teranishi's (*14*). Preparative-scale gas

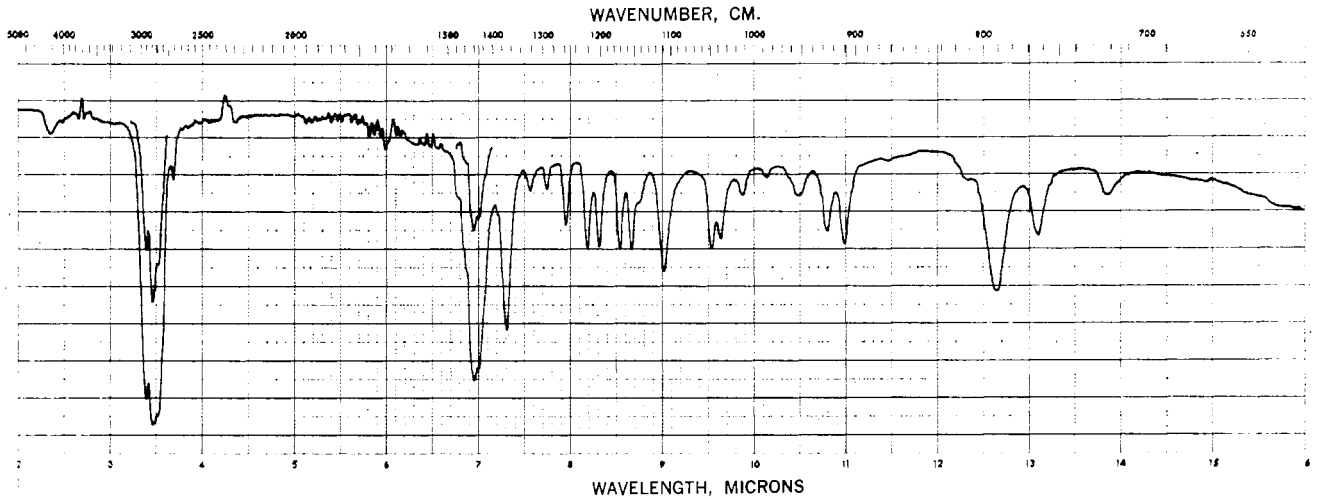

Figure 5. Infrared spectrum of terpinolene

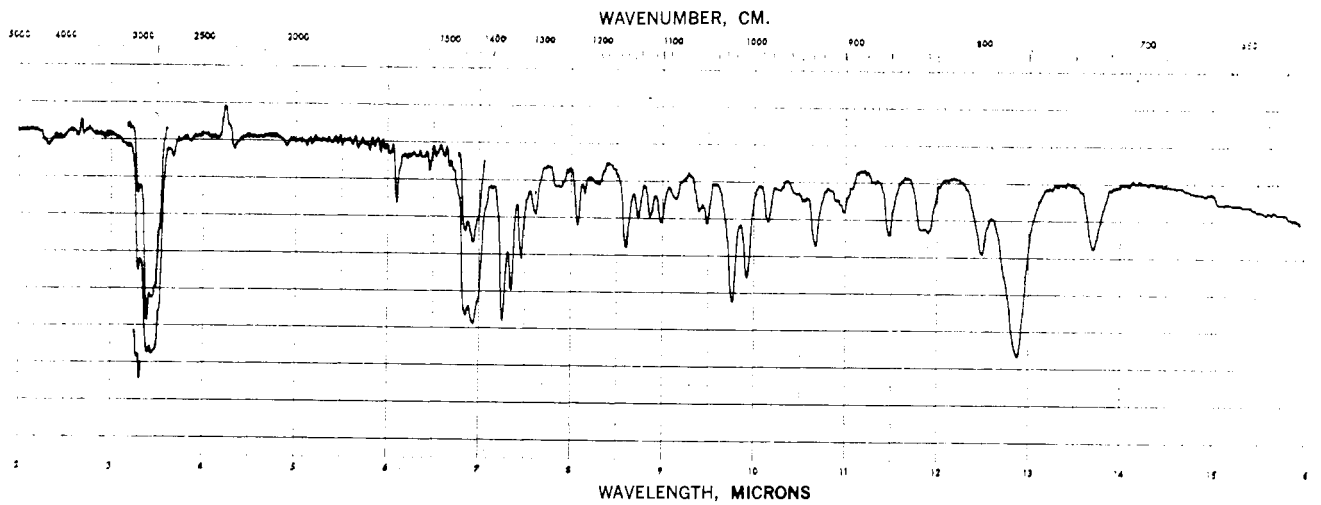

Figure 6. Infrared spectrum of α-thujene

chromatography failed to resolve the component thought to be α-terpinene from limonene, and a two-component mixture that was approximately 40% α-terpinene and 60% limonene was collected. The infrared spectrum of this mixture was predominantly characteristic of limonene, but the relatively strong absorption at 12.1 microns was characteristic of α-terpinene and not of limonene. Also, ultraviolet absorption at 265 mμ was consistent with that reported by O'Connor and Goldblatt (*9*) for α-terpinene.

Terpinolene. Terpinolene has been reported by Guenther (*4*) in *Manila clemi* and Monterey cypress oils. Those oils were not available to the authors. None of the essential oils examined by Ikeda *et al.* showed a relatively large amount of terpinolene. A commercial sample of terpinolene was obtained, but the major component was identified as α-pinene (70%). A fraction having the retention expected to be terpinolene on the basis of retention data reported by Ikeda *et al.* (*6*) was only 6% of the monoterpene hydrocarbons. The terpinolene distillate was redistilled in an effort to concentrate the higher-boiling monoterpene hydrocarbons. The residue of the second distillation consisted of 40% of what was hoped to be terpinolene. The ultraviolet spectrum was consistent with the unconjugated structure, and the infrared spectrum (Figure 5) was in reasonable agreement with that reported by O'Connor and Goldblatt (*9*) but was not in agreement with the spectrum given in the Sadtler tables (*11*). It matched precisely, however, with Teranishi's spectrum of terpinolene (*14*).

A fraction having the same retention was isolated in the previously described silica gel isomerization of limonene. The infrared spectrum of this compound was also identical to that of terpinolene.

β-Phellandrene. Guenther (*4*) reported that β-phellandrene occurs in numerous oils of star anise, water fennel, angelica seed, African ginger, Japanese pepper, *Pinus contorta*, Canada balsam, lemon, Seychelles cinnamon, *Schinus molle* L., *Eucalyptus amygdalina*, and Italian seafennel. Ikeda *et al.* (*6*) reported its presence (12.7%) in oil of cubeb. The authors' examination of cubeb oil showed a fraction of corresponding retention time to be present in amounts of 6%. A commercial sample of α-phellandrene was 71% pure. Ten per cent of the sample was a fraction having a retention time expected to be β-phellandrene according to retention data published by Ikeda *et al.* (*6*). This compound was isolated, and its absorption at 232 mμ would be consistent with the structure of a conjugated hydrocarbon having one double bond in a ring (*9*). A strong absorption at 11.45 microns was also consistent with this structure.

A doublet at 6.12 and 6.28 microns was in agreement with Teranishi's spectrum of β-phellandrene. However, absorption at 10.15 microns and in other areas of the fingerprint region indicated the possibility of impurities.

α-Thujene. α-Thujene has been identified as the main constituent in the

terpene fraction of oil distilled from the gum oleoresin of *Bosuellia serrata* Roxb. and of oil of the leaves of the Formosa Hinoki tree. Those oils were not available to us. Ikeda reported cedar leaf oil (thuja oil) to contain 13.0% α-thujene and oil of cubeb to contain 13.2% α-thujene (*6*). α-Pinene was reported to occur in these oils in quantities of 15.6 and 12.1%. These oils were examined as possible sources of α-thujene, but we failed to resolve α-thujene from α-pinene on the preparative gas chromatograph. [Klouwen and Ter Heide recently reported that α-thujene could be separated from α-pinene on Apiezon L (*7*). This has since been confirmed by the authors.] A sample of α-thujene isolated by Ikeda from *Eucalyptus dives* was obtained from Teranishi. The infrared spectrum (Figure 6) is in agreement with that published in the Sadtler tables; the absorption observed in the ultraviolet is consistent with this structure.

Acknowledgment

The authors are grateful to Roy Teranishi, Western Regional Research Laboratory, Albany, Calif., for access to his library of infrared spectra. They thank R. A. Bernhard, of this department, for samples of orange and lemon oil.

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Received for review February 28, 1964. Accepted May 29, 1964.

ASTRINGENCY PRINCIPLES

Changes in Phenolic Content in Persimmons during Ripening and Processing

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Changes in concentration of phenolic substances extractable with methanol and aqueous methanolic solution were compared with histochemical observations and organoleptically observed changes in astringency. Decrease in extractability of phenolics by methanol accompanied loss in fluidity of cellular tannins and decrease in astringency. Oxidation was found to be responsible for loss in astringency on air drying of sliced persimmons or high speed blending. Pureeing and freezing of astringent tissue resulted in loss of astringency and decrease in phenolics even in presence of added ascorbic acid or sulfite. The leucoanthocyanin isolated from methanolic extracts of astringent tissue contained both leucodelphinidin and leucocyanidin, apparently present together in the molecule.

THE disappearance of astringency with ripening in persimmon fruit is one of the most pronounced ripening changes known. The nature of the changes occurring during loss in astringency has been investigated by botanists, plant physiologists, and chemists, but our knowledge of the composition and structure of the phenolic compound involved and of changes in its molecular structure during ripening is still incomplete.

Until very recently but few quantitative chemical data were available on changes in tannin content during ripening of astringent and nonastringent varieties. The earliest studies reported were those of Bigelow *et al.* (*2*) in the United States. Only a few of the papers published in Japan give data on "tannin" content (*33*); and even in the more recent work tannins are determined by the nonspecific acid permanganate titration procedure (*18, 19*). While it is now fairly well established that the organic tannin-like astringents of food are related to leucoanthocyanins (*1, 30*), their molecular structure is still unknown. Ito and Oshima (*14*) reported the isolation of a leucoanthocyanin identified as leucodelphinidin-3-glucoside as the main component of persimmon tannin. Their preparation was astringent at a concentration of 20 mg. per 100 ml., but apparently was of high molecular weight, because it was not chromatographically mobile in the usual phenolic solvents. Siegelman and Craft (*26*), who also reported isolation of a partially purified persimmon leucoanthocyanin, estimated its molecular weight to be 50,000.

Lloyd (*16*) first observed that the tannin present in specialized tissue cells is fluid in unripe astringent persimmons and easily spreads over cut surfaces, but in ripe fruit the tannin cells no longer