The importance of water in the vacuum distillation method may be related to the failure of DCM to extract the NDMA from solid malt, while the direct aqueous extraction was successful. The low moisture content of dried malted barley and the polarity of NDMA may allow NDMA binding to some molecular constituent in the malt. Apparently nonpolar DCM fails to release the bound NDMA, whereas the addition of a highly polar solvent such as water is necessary for efficient release. Further work is underway to elucidate the nature of the NDMA binding.

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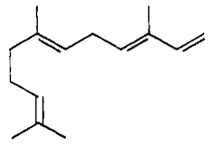
Characterization of a New Citrus Component, *trans,trans*-α-Farnesene. Isolation of α-Farnesene Isomers from Dehydration of Farnesol

trans,trans-α-Farnesene, which had never been found in citrus, was identified as a component in cold-pressed Valencia orange oil and distilled lime oil. Although *trans*-β-farnesene is a relatively common food constituent, only rarely has an α-farnesene isomer been identified in any food. Dehydration of farnesol and separation of the crude product by gas chromatography afforded an authentic sample of *trans,trans*-α-farnesene, as well as the other three α-farnesene isomers and the two β-farnesene isomers. This dehydration reaction had been reported to yield *trans*-β-farnesene as the only isolatable farnesene isomer.

Economic importance of citrus oils and food safety for the consumer depend upon composition of natural flavor components being determined as thoroughly as possible. Therefore, research efforts are continuing to identify and evaluate new constituents of citrus flavor fractions. The many studies identifying citrus components from each type of fruit were reviewed by Shaw (1977a,b). *trans*-β-Farnesene [(*E*)-7,11-dimethyl-3-methylenedodeca-1,6,10-triene] has been identified in cold-pressed orange oil and lemon oil (Shaw, 1977a) and has been quantitated in orange oil (Shaw and Coleman, 1974), but none of the other five possible isomers have been identified in any citrus oil or essence. Anet (1970) has synthesized all six isomers of α- and β-farnesene by acid-catalyzed dehydration of nerolidol and has assigned configurations to the two β-farnesenes and the four α-farnesenes thus found.

In the current study, the volatile compound, *trans,trans*-α-farnesene (1) [(3*E*,6*E*)-3,7,11-trimethyldodeca-1,3,6,10-tetraene] was isolated from distilled lime oil and



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from cold-pressed Valencia orange peel oil. It is being reported for the first time as a constituent of citrus. Acid-catalyzed dehydration of farnesol afforded an authentic sample of *trans,trans*-α-farnesene, as well as other α-farnesene isomers. This dehydration reaction has been reported to yield *trans*-β-farnesene as the only isolatable farnesene isomer (Naves, 1966; Brieger et al., 1969).

EXPERIMENTAL SECTION

Separation Procedures. Samples of distilled Mexican lime oil and cold-pressed Valencia orange oil were analyzed by gas–liquid chromatography (GLC) on packed columns on a Perkin-Elmer Model 900 gas chromatograph equipped with a thermal conductivity detector, using 0.10 in. i.d. × 20 ft stainless steel columns packed with either 10% Carbowax 20M or 10% UCW-98 on 60–80 mesh Gas-Chrom P. The following operating conditions were used: helium flow, 36 mL/min; injection port temperature, 250 °C; detector temperature, 275 °C; column temperature, 80–210 °C at 2 °C/min. Individual compounds were collected as they were eluted from the polar (20M) column and reinjected onto the nonpolar (UCW-98) column for further purification when necessary. Separations on a glass capillary column (60 m) coated with Carbowax 20M were carried out with a Hewlett-Packard Model 5840A gas chromatograph equipped with an injection port splitter (100:1). Operating conditions were as follows: injection port temperature, 250 °C; FID detector temperature, 300

Table I. Products from Acid-Catalyzed Degradation of Farnesol

product	GLC t_r, min: packed[a]	GLC t_r, min: capillary[a]	GLC area, %[b]	λ_{max}, nm[c]
cis-β-farnesene	55.3	34.3	11.4	226
trans-β-farnesene	58.0	35.4	18.7	226
cis,cis-α-farnesene[d]	60.9	36.4	3.8	234[e]
cis,trans-α-farnesene	60.9	36.7	1.7	
trans,cis-α-farnesene	61.6	37.4[f]	11.9	233
trans,trans-α-farnesene	64[g]	37.9[f]	17.4	233
β-bisabolene	64[g]	38.4	10.6	
cis-α-bisabolene	66.5	39.7	17.5	

[a] Carbowax 20M column. [b] Determined on the 60M glass capillary column coated with Carbowax 20M. [c] In absolute EtOH, sample size too small for accurate determination of extinction. [d] Tentative identification based on retention time. [e] UV determined on the mixture only. [f] Peak identity confirmed by enrichment with sample isolated and identified from packed column. [g] Separated by rechromatography on the packed nonpolar column.

°C; He flow 21 cm/s; column temperature program, 85 °C for 10 min and then raised to 190 °C at 4 °C/min.

Comparison of infrared and/or mass spectra with those of authentic samples led to positive identifications. Mass spectra were obtained on a Hewlett Packard 5985A GC/MS data system and infrared spectra were obtained on a Perkin-Elmer Model 727B infrared spectrophotometer with samples as oil films.

Dehydration of Farnesol. A mixture of 21 g of farnesol (Chemical Samples Co., Columbus, OH) and 7.5 g of potassium bisulfate was distilled for 1 h at ca. 100 °C and 1.5 mmHg to afford about 11.5 g of crude product mixture that was used for GLC separation.

RESULTS AND DISCUSSION

A volatile compound not reported previously as a citrus component, *trans,trans*-α-farnesene, was found in distilled lime oil and in cold-pressed Valencia orange oil. On the basis of GLC peak areas (polar column) the content of *trans,trans*-α-farnesene in the distilled lime oil sample was estimated to be 1.4%. The quantity present in Valencia orange oil was too small to be estimated, but it was less than the 200 ppm reported for the more abundant isomer, β-farnesene, found in orange oil by Shaw and Coleman (1974).

Authentic *trans,trans*-α-farnesene was isolated as a product of the acid-catalyzed dehydration reaction of farnesol. This reaction was reported previously to provide a mixture of at least 12 components, including *trans*-β-farnesene, although none of the four possible α-farnesene isomers or *cis*-β-farnesene could be isolated (Naves, 1966; Brieger et al., 1969). However, Naves (1966) did suggest from ultraviolet spectral evidence that α-farnesene was present in the mixture. We found the reaction mixture from dehydration of farnesol to consist of mainly the eight components listed in Table I in order of their GLC retention times on a packed Carbowax 20M column. Five later-emerging components were also detected, but were not identified. The major α-farnesene isomer, *trans,trans*-α-farnesene, was not separated from β-bisabolene on the packed polar column, but rechromatography of the two-component mixture on a nonpolar column afforded a pure sample of *trans,trans*-α-farnesene. A polar capillary column separated these two components almost completely.

We assigned configurations to the α-farnesene isomers that we isolated (Table I) largely on the basis of comparisons we made of their relative GLC retention times on a polar (Carbowax 20M) column and their infrared spectra with those listed by Anet (1970) for all six isomers of α- and β-farnesene. Unlike Anet, we were able to separate the cis,trans-α and trans,cis-α isomers partially on a packed polar GLC column and completely on a capillary column. The intensities of the two diagostic infrared bands at 1599–1610 and 1635–1648 cm^{-1} in conjunction with relative GLC retention times were very helpful in assigning the relative configurations of the α-farnesene isomers. The ultraviolet maxima at 233–235 nm did not help us distinguish the various α-farnesene isomers because the differences in peak absorption maxima were too small to be useful. Although we were unable to positively identify the fourth possible α-farnesene isomer (*cis,cis*-α-farnesene), rechromatography of *cis,trans*-α-farnesene from the packed column in Table I on a nonpolar column showed a partly resolved component with a slightly shorter retention time, and that may have been the cis,cis-α isomer. On a polar glass capillary column, this component was clearly separated and was assigned the structure listed in Table I on the basis of its GLC retention time. An insufficient quantity of this compound was isolated for complete characterization.

Only rarely have any α-farnesene isomers been reported as constituents in food (vanStraten and deVrijer, 1973). Murray (1969) found, in a 300:1 ratio, *trans,trans*-α-farnesene and a second isomer to which he assigned the structure *trans,cis*-α-farnesene as constituents of the natural coating on apples. Later, Anet (1970) used spectral evidence to assign the structure *cis,trans*-α-farnesene to the minor isomer found in apples. Naya and Kotake (1970) reported an α-farnesene as a constituent of hops but did not designate which isomer they had isolated.

The flavor contribution of this new citrus constituent was not thoroughly assessed because only trace quantities were isolated. However, the aroma of *trans,trans*-α-farnesene, like that of many sesquiterpenes, is characterized by a heavy, floral or woody note that is important to the full-bodied aroma of many oils.

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