are summarized in Table III. It can be seen that 2-methylpropionic, 2-methylbutyric, and 3-methylbutyric acids increase strongly during the storage of hops. They are derived by degradation of cohumulone, adhumulone, and humulone and are responsible for the unpleasant aroma of stored hops. Pentanoic, hexanoic, octanoic, 2-hexenoic, and 3-hexenoic acids are formed by oxidation of fatty acids. Most of the other compounds may be produced by hydrolytic cleavage of the corresponding methyl esters.

The results show that the composition of hop aroma constituents changes considerably during storage. Terpene and sesquiterpene hydrocarbons are decreased by polymerization and oxidation. To some extent they are transformed into hydrophylic components such as epoxides and alcohols, which may be easily transferred to beer. These reactions are comparable to processes during the kettle hop boiling. In addition, components such as aldehydes and fatty acids, which are known as off-flavor constituents in beer, are formed.

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Synthesis of Nootkatone from Valencene

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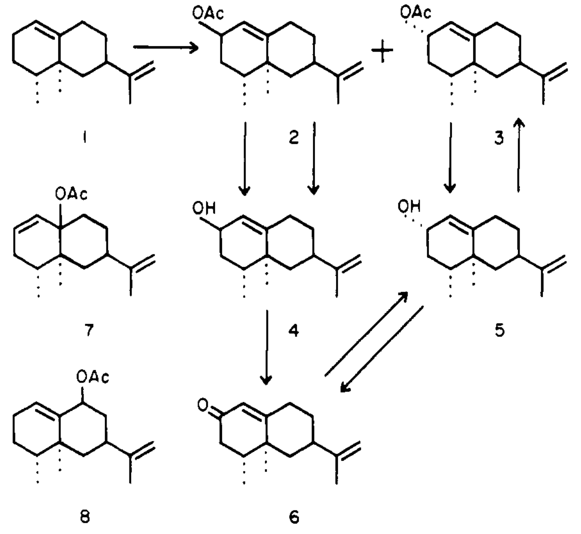
Valencene (1) was converted by a three-step process to nootkatone (6) in 47% overall yield. Oxidation of 1 with *tert*-butyl peracetate afforded a mixture of esters 2 and 3 in 98:2 ratio (a/e); the esters were hydrolyzed to the corresponding alcohols 4 and 5 and the alcohols were oxidized with chromic acid to 6. The conformations of alcohol 5 and ester 3 were determined by reduction of 6 with metal hydride to 5 and acetylation of alcohol 5 with acetic anhydride/pyridine to ester 3. Thus, the *tert*-butyl peracetate oxidation of 1 to nootkatone (6) in good yield affords an alternate commercial method that provides intermediate alcohols and acetate esters with potentially valuable flavor properties and involves a less hazardous preparation of nootkatone than the presently used oxidation with chromate.

Nootkatone (**6**), an important flavoring constituent of grapefruit, is used commercially to flavor soft drinks and other beverages (Macleod and Buigues, 1964) and in perfumes. Nootkatone has been prepared from the sesquiterpene valencene (**1**) by oxidation with *tert*-butyl chromate in crude yield of 67% (Hunter and Brogden, 1965). However, this reaction is explosive if not conducted at room temperature or lower. We report a three-step synthesis of nootkatone from valencene in 47% overall yield and discuss the reaction intermediates involved. The method provides intermediate alcohols and acetate esters with potentially valuable flavoring properties and does not involve the use of *tert*-butyl chromate.

When **1** was oxidized with *tert*-butyl peracetate, the axial acetate **2** was the major reaction product (Scheme I); axial esters have been the major products of similar perester oxidation reactions (Beckwith and Phillipon, 1976; Wilson and Shaw, 1975). A small amount (ca. 2%) of the equatorial acetate **3** was also isolated by preparative thin-layer chromatography (TLC). Alternative allylic acetates **7** and **8**, which are possible products of this reaction, were not found, presumably because their formation was hindered sterically.

Scheme I



To determine the conformation of the predominant acetate **2** we converted it to alcohol **4** by treatment with lithium aluminum hydride. Comparison showed that **4**

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differed from the known equatorial alcohol nootkatol (**5**), obtained by metal hydride reduction of nootkatone. Thus, acetate **2** was the axial isomer. The minor acetate **3** afforded nootkatol (**5**) upon treatment with lithium aluminum hydride. Both alcohols **4** and **5** afforded nootkatone upon oxidation with chromic acid.

Konst et al. (1975) reduced nootkatone with bis(2-methoxyethoxy)aluminum hydride and had stated that, from NMR evidence, their nootkatol (**5**) apparently contained about 20% of the axial isomer, but they did not separate the isomers. In our experiments sodium borohydride and sodium bis(2-methoxyethoxy)aluminum hydride reductions of nootkatone afforded alcohols **4** and **5** in 1:1 ratio as determined by preparative TLC. The identity of **5** was confirmed by comparison of its NMR spectrum with that published by Konst et al. (1975).

The identity of the minor acetate **3** isolated in the perester oxidation of valencene was confirmed when it was found to be the same as the product from acetylation of nootkatol (**5**) derived from reduction of nootkatone.

In the oxidation of valencene, the yield of nootkatone could be increased if the intermediate esters and alcohols are not isolated. With 2 equiv of *tert*-butyl peracetate, the yield of nootkatone was 47% overall, and no starting valencene was recovered. When 1 equiv of perester was used, the yield of nootkatone was only 20%, and 34% starting valencene was present as determined by GLC analysis. Neither the esters nor the alcohols could be recovered in attempted GLC of the crude mixtures, even when a glass column and a glass-lined injection port were used.

The isomeric alcohols and acetate esters isolated as intermediates in this study all had strong aroma properties. The axial alcohol (**4**) and acetate (**2**) had stronger aromas than the corresponding equatorial isomers. Acetates **2** and **3** had woody, cedar-like aromas and alcohols **4** and **5** had grapefruit-like aromas. The axial isomer **4** had a pronounced grapefruit-like aroma, whereas the equatorial isomer had a combined woody- and grapefruit-like aroma that was weaker than that of **4**. Konst et al. (1975) reported a grapefruit-like aroma for **5**, but did not evaluate the aroma of **4**.

EXPERIMENTAL SECTION

Infrared spectra of thin liquid films were obtained with a Perkin-Elmer Model 137 infrared spectrometer. NMR spectra were determined with a Varian EM-360 spectrometer; $CDCl_3$ was the solvent and Me_4Si was the internal standard. Ester and alcohol mixtures were separated by preparative TLC on 1000 μ silica gel HF_{254} plates with hexane-acetone mixtures (99:1, v/v, solvent 1; 90:10, v/v, solvent 2) as the developing solvents. Nootkatone was separated and quantitated by use of an F&M Model 700 gas chromatograph, equipped with an 0.20 in. × 20 ft stainless steel column packed with 20% Carbowax 20M on 60–80 mesh Gas-Chrom P and a thermal conductivity detector at 275 °C. Temperature was programmed from 100 to 220 °C at 2 °C/min, and He flow rate was 100 mL/min. The injection port temperature was 245 °C. Reaction product percentages were determined from peak areas (height × width at half-height) of GLC curves for crude nootkatone mixtures.

Preparation of Acetates 2 and 3 from Valencene (1). To 20.4 g (0.10 mol) of valencene (**1**) (determined to be 90% pure by GC) and 0.5 g of cuprous bromide, 38 g (0.20 mol) of 75% *tert*-butyl peracetate (ICN Pharmaceuticals, Inc., Plainview, N.Y.) was added dropwise in 1 h as the mixture was stirred. The reaction mixture was heated in an oil bath at 82 °C for 24 h, cooled, mixed with 100 mL of diethyl ether, and then filtered into a separatory funnel. The filtrate was washed four times with 25-mL portions of saturated sodium carbonate and then dried over magnesium sulfate. The solvent was removed, leaving a mixture of esters as an oily residue. Distillation of the oily residue yielded a mixture of compounds **2** and **3** [bp 115–125 °C (0.05 mmHg)], and the infrared spectra indicated the mixture contained mostly compound **2**.

The crude reaction mixture (oily residue) was separated by preparative TLC (solvent 1) into compounds **2** and **3** in a ratio of about 98:2. Compound **2**: R_f 0.19; IR (thin film) 2900, 1725, 1460, 1450–1430 doublet), 1360, 1240, 1145, 1045, 1018, 985, 948–935 (shoulder), 928, 890, 830, 758 cm^{-1}; NMR ($CDCl_3$) δ 0.90 (broad, 6 H, C—CH_3), 1.70 (s, 3 H, C=C—CH_3), 2.01 (s, 3 H, $COCH_3$), 4.67 (s, 2 H, C=CH_2), 5.27 (m, 1 H, C=CH—). Compound **3**: R_f 0.15; IR (thin film) 2900, 1725, 1650, 1458, 1440 (shoulder), 1370, 1240, 1195, 1158, 1140 (shoulder), 1125, 1070, 1028, 980, 968, 952, 935, 895, 845, 819 cm^{-1}; NMR ($CDCl_3$) δ 0.98 (d, 3 H, CH—CH_3, J = 4 Hz), 1.00 (s, 3 H, CH_3), 1.63 (s, 3 H, C=CH_3), 2.0 (s, 3 H, $COCH_3$), 4.63 (s, 2 H, C=CH_2), 5.27 (s, broad, 1 H, C=CH—).

Reduction of Acetates 2 and 3 to Alcohols 4 and 5. To 100 mg of **2** in 5 mL of tetrahydrofuran (THF) was added 25 mg of $LiAlH_4$. After 2 h the reaction was quenched with water, the mixture filtered, and the filtrate dried over magnesium sulfate. The mixture was concentrated, and the residue was separated by preparative TLC (solvent 2). Compound **4** at R_f 0.28 was obtained in about 50% yield: IR (thin film) 3300, 2890, 1640, 1450–1430 (doublet), 1370, 1145, 1120, 1078 (shoulder), 1062, 1050, 1010, 932, 890, 838, 770 cm^{-1}; NMR ($CDCl_3$) δ 0.90 (s, 3 H, C—CH_3), 0.96 (d, 3 H, C—CH_3, J = 4 Hz), 1.72 (s, 3 H, C=C—CH_3), 4.07 (s, 1 H, CH—O), 4.67 (s, 2 H, C=CH_2), 5.40 (m, broad 1 H, C=CH). Similarly, acetate **3** was treated with $LiAlH_4$ to afford nootkatol (**5**), identical by infrared spectrum and TLC R_f to **5** prepared from nootkatone below.

Reduction of Nootkatone (6) to Alcohols 4 and 5 (Nootkatol). To 100 mg of crude nootkatone in EtOH was added 1 mL of a solution containing about 20 mg/mL of $NaBH_4$. The mixture was allowed to stand 4 h, then 25 mL of saturated NaCl solution was added and the mixture extracted four times with 25-mL portions of diethyl ether. The combined ether solution was dried over magnesium sulfate and concentrated. The reaction afforded **4** (R_f 0.15) and **5** (R_f 0.25) in 1:1 ratio, as shown by preparative TLC (solvent 2). Compound **5**: IR (thin film) 3300, 2890, 1640, 1450, 1435 (shoulder), 1375, 1350 (shoulder), 1210, 1185, 1150, 1120, 1100 (shoulder), 1080, 1065, 1050, 1030, 980, 965, 890, 845, 818, cm^{-1}; NMR ($CDCl_3$) δ 0.96 (d, 3 H, CH—CH_3, J = 3 Hz), 0.98 (s, 3 H, CH_3), 1.67 (s, 3 H, C=CH_3), 2.29 (s, 1 H, C—OH disappears with D_2O), 4.10 (s, broad, 1 H, CH—O), 4.67 (s, 2 H, C=CH_2) (Konst et al., 1975).

Similarily, 1.0 g of nootkatone (**6**) was reduced with 1.3 g of sodium bis(2-methoxyethyl)aluminum hydride according to Konst et al. (1975) to afford alcohols **4** and **5** (nootkatol) in a 1:1 mixture as shown by preparative TLC (solvent 2).

Oxidation of Alcohols 4 and 5 to Nootkatone (6). Fifty milligrams of either alcohol **4** or **5** was spotted on a preparative TLC plate and 100 μL of potassium dichromate in aqueous sulfuric acid (Jones reagent; Bowder et al., 1946) was spotted on top of the alcohol at the origin. The TLC plate was dried and developed in solvent 2, and the band at the R_f corresponding to nootkatone was scraped off and eluted. The product, formed in about 50%

yield (determined by GLC area percent values), was shown to be nootkatone by comparison of its infrared spectrum and GLC retention time with those for an authentic sample.

Acetylation of Nootkatol (5) to Acetate 3. To 100 mg of compound **5** was added 300 μL of a 2:1 mixture of acetic anhydride and pyridine. The mixture was allowed to stand overnight and was then separated by preparative TLC in solvent 1. Compound **3** was afforded in about 80% yield.

Conversion of Ester Mixture 2 and 3 to Nootkatone (6). To 12 g of crude ester mixture in 100 mL of diethyl ether was added 50 mL of sodium hydroxide solution (58 g of NaOH in 500 mL of 1:1 methanol–water), and the solution was stirred overnight. The aqueous layer was separated and extracted two times with 25-mL portions of diethyl ether, and the ether extracts were added to the original ether layer.

The combined ether solution was dried over magnesium sulfate and concentrated to afford 9.0 g of crude alcohol mixture. Where the crude mixture was separated by preparative TLC (solvent 2), alcohol **4** was recovered in about 65% yield.

The crude alcohol mixture in 100 mL of diethyl ether was treated with 35 mL of aqueous chromic acid and the mixture stirred overnight. The layers were then separated, the aqueous layer was extracted two times with diethyl ether, and the extracts were added to the original ether solution. The combined ether solution was then dried over magnesium sulfate and concentrated to afford 6.6 g of crude product. Qualitative and quantitative GLC analysis of the crude mixture showed it contained 85.5% nootkatone (**6**) assuming the sample was 100% GLC volatile (47% overall yield from valencene).

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Quantitative Composition of Cold-Pressed Grapefruit Oil

Charles W. Wilson, III* and Philip E. Shaw

The major volatile components of Florida cold-pressed white grapefruit oil were quantitatively analyzed by gas chromatography. Corrected weight percentages were determined from response factors and the precentages of nonvolatiles in the whole oil. Of the 24 identified constituents, 19 were quantitated. Data for only 9 of the 19 could be compared with literature values, but the agreement was generally good. The octanal to decanal ratio was slightly greater than 1. Quantities of the two major esters, octyl and neryl acetates, are reported for the first time.

Reliable quantitative information on individual components of cold-pressed grapefruit oil is scarce relative to comparable data on orange, mandarin, and lemon oils (Shaw, 1977). With such data on grapefruit, the contribution of individual compounds to the total flavor profile of grapefruit oil could be assessed, the chemotaxonomy of grapefruit hybrids could be studied, and entomologists may be better able to study reasons for survival of insect larvae in grapefruit peel.

Reports to date on quantitative analysis of grapefruit oil constituents have emphasized the aldehydes because they are major contributors to citrus oil flavors. Yokoyama et al. (1961) compared colorimetric methods for quantitating citral in various citrus oils. Stanley et al. (1961) and Braddock and Kesterson (1976) quantitated the major aldehydes in citrus oils, but the aldehyde values of Stanley et al. (1961) could only be related to total oil by use of an estimated value for total aldehydes present (Shaw, 1978).

Other studies on grapefruit oil quantitation involved injection of the whole oil onto a gas chromatographic (GLC) column (Ashoor and Bernhard, 1967; Attaway et al., 1967); but in one of these (Attaway et al., 1967) the workers used relative peak heights to determine the relative percentage of each component rather than the more accurate quantitation based on peak areas. Ikeda et al. (1962) quantitated the major hydrocarbons in grapefruit oil by GLC after a preliminary separation by thin-layer chromatography (TLC). However, in none of those studies were the GLC response factors or the considerable number of nonvolatiles present in grapefruit oil taken into account.

In the current study, whole cold-pressed grapefruit oil was directly injected onto a GLC column, and the weight percentage of each major oil component was determined from response factors and the precentage of nonvolatiles retained by the column.

EXPERIMENTAL SECTION

Cold-pressed white grapefruit oil from Duncan and Marsh seedless grapefruit that had been stored several days at 0 °C or less (winterized) was obtained from a local processor in November 1977 and stored at 4 °C until used. Authentic samples of individual compounds for response

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