ley, CA, for the authentic samples used in this work.

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Composition of Essence Oil from Overripe Oranges

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Analysis of essence oil from overripe oranges yielded 41 compounds, 14 of which are being reported for the first time as components of orange essence oil. We identified none of the phenolic ethers that had been found in products from oranges treated with abscission chemicals and judged to contribute an overripe flavor. A sensory panel preferred essence oil from ripe fruit (control) to that from overripe fruit, but showed no preference between control oil and a blend of oils from control and overripe fruit. Essence oil from overripe oranges contained more valencene than that from control fruit. Thus, it appears it is the best available source of valencene, a desirable flavor-related compound.

Recent studies by Moshonas et al. (1976, 1977) showed that abscission agents used to loosen citrus fruit prior to mechanical harvesting affect the quality of the processed orange juice and cold-pressed essential oil. In 1978, Moshonas and Shaw isolated and identified six phenolic ethers present in fruit that had been treated with abscission chemicals. The compounds were shown to be unnatural components formed by a change in metabolic pathways brought about by the chemicals. Since a majority of an expert taste panel indicated that products from oranges treated with abscission chemicals had an overripe flavor (Moshonas et al., 1976, 1977), we undertook to determine whether the phenolic ethers are synthesized when the natural metabolic processes are allowed to continue beyond the normal harvesting period for oranges.

The primary byproduct obtained during the concentration step in the preparation of frozen orange juice concentrate is aqueous essence. Part of the essence is subsequently returned to the concentrate to give it a fresh orange aroma. A second byproduct, essence oil, separates from the aqueous essence and is also used as a flavoring agent. Essence oil contains most of the oil-soluble flavoring components of orange juice. Thus, if present in overripe oranges, the phenolic ethers should be recovered in the essence oil of those fruit.

Orange essence oil has been analyzed by several workers. In 1965, Hunter and Brogden analyzed the hydrocarbons in essence oils. Coleman et al. (1969) compared composition of mid- and late-season orange essence oils and found quantitative and qualitative differences. Veldhuis et al. (1972) compared composition of orange essence oil with that of an aroma oil prepared by distillation of aqueous discharge from centrifugation of orange peel oil. Shaw and Coleman (1971) analyzed the highly volatile compounds distilled from essence oil and Coleman and Shaw (1971) reported quantitative and qualitative analysis of essence oil constituents. However, in none of these studies was essence oil from overripe fruit analyzed.

The current study reports major components of essence oil from overripe Valencia oranges, including 14 compounds not found previously in essence oil, and compares the composition of essence oils from overripe and normal fruit. An aroma panel was used to compare aromas of essence oils from overripe fruit and normal fruit and to determine affects of blending essence oils from overripe fruit with that from normal fruit.

EXPERIMENTAL SECTION

Essence oils from Valencia oranges harvested after the regular season (late July) and from Valencia oranges harvested in March, and known to have good flavor and aroma qualities, were obtained from Redd Laboratories, Safety Harbor, FL, and stored at 5 °C.

Whole Oil Analyses. Each of the essence oils was injected directly into the gas chromatograph (GLC) for separation and purification of major constituents so that they could be identified and quantitated. A Hewlett-Packard Model 3380-A computing integrator coupled to the gas chromatograph measured GLC peak areas for the quantitative work.

Separation Procedure. Essence oil from overripe Valencia oranges (84.5 g) was distilled at a bath temperature of 35 °C and 0.5 mmHg from a rotary evaporator until most of the hydrocarbons (99% limonene) were removed (81.5 g). A liquid nitrogen trap located between the receiver and vacuum pump contained 0.4 g of liquid. The distillation residue (2.6 g) was transferred into an ice–water jacketed column (1 × 15 in.) containing 60/80 mesh Florisil (Fisher Scientific Co.) deactivated with 6% water (Lund and Coleman, 1977). Fractions were eluted with 300 mL

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Table I. Orange Essence Oil Components

alcohols	esters
cis-carveol[a]	ethyl acetate
trans-carveol[a]	ethyl butyrate
citronellol[a]	ethyl propionate
decanol[a]	methyl butyrate
dodecanol[a]	1,8-*p*-menthadien-9-yl acetate[a,b]
elemol[a,b]	octyl acetate[a,b]
geraniol[a,b]	hydrocarbons
linalool[a]	β-caryophyllene[a]
intermedeol[a,b]	α-copaene[a]
1,8-*p*-menthadien-9-ol[a,b]	β-copaene[a]
cis-2,8-*p*-menthadien-1-ol[a]	β-cubebene[a]
trans-2,8-*p*-menthadien-1-ol[a]	*p*-cymene[a]
nerol[a,b]	β-elemene[a]
nonanol[a]	farnesene
octanol[a]	heptane
α-terpineol[a]	hexane
undecanol	α- and β-humulene
aldehydes	limonene[a]
acetaldehyde	myrcene[a]
decanal[a]	nootkatene[a,b]
dodecanal[a,b]	α-pinene[a]
geranial[a]	sabinene
octanal[a]	epi-α-selinene[a,b]
perillaldehyde[a]	valencene[a]
α-sinensal[a,b]	miscellaneous
β-sinensal[a,b]	1,1-diethoxyethane
ketones	*trans*-linalool oxide[a,b]
acetone	
carvone[a]	
ethyl vinyl ketone	
nootkatone[a,b]	
piperitenone[a]	

[a] Constituents of orange essence oil identified in this study. [b] Constituents of orange essence oil being reported for the first time.

of hexane to remove the remaining hydrocarbons, 300 mL of a 2:1 hexane–ethyl ether solution to concentrate carbonyl-containing compounds, and 300 mL of absolute ethanol to remove the remaining compounds. Gas chromatographic analyses of these fractions, the hydrocarbon distillate, the material in the liquid nitrogen trap, and the whole oil were carried out on a Perkin-Elmer Model 900 gas chromatograph equipped with a thermal conductivity detector and a 0.10-in. i.d. × 20 ft column packed with either 10% Carbowax 20M or with 10% UCW-98 on 60/80 mesh Gas-Chrom P. For all runs the injection port temperature was 275 °C and detector temperature was 290 °C. Oven temperature was programmed from 80 to 210 °C at 2 °C/min and helium flow was 30 mL/min. Individual compounds were collected as they were eluted from the GLC and were positively identified by comparison of their infrared spectra, mass spectra, and retention times with those of authentic samples.

Aroma Panel Tests. Paired comparison tests on essence oils from control vs. overripe fruit were conducted using equal volumes of the two oils in identical 1 dram vials at room temperature (Shaw et al., 1971). A well-ventilated room with subdued lighting and individual booths was used for all tests. The aroma panel consisted of 12 experienced members, each of whom made two determinations. Panelists were asked to compare the essence oils from the ripe and overripe oranges and give their preference. To minimize fatigue, which is common in aroma tests with oil samples, 30 min was allowed between the two presentations.

RESULTS AND DISCUSSION

Comparison of the major compounds in the essence oils from ripe and overripe oranges showed quantitative but not qualitative differences. By a thorough analysis of overripe essence oil, we isolated and identified 14 compounds not previously reported as components of orange essence oil. However, we did not find any of the phenolic ethers found in products made from oranges treated with abscission chemicals.

Table II. Aroma Preference Tests of Valencia Orange Essence Oils (24 Judgements)

samples	preference for: control	preference for: overripe	preference for: blend	confidence level, %
control vs. overripe	19	5		99
control vs. 1:9[a] blend	13		11	ns[b]

[a] Overripe/9 control. [b] Not significant.

Orange essence oils differ in composition, flavor, and aroma from cold-pressed orange oils. Table I lists all compounds isolated and identified as constituents of orange essence oil at our laboratory. The presence of the more volatile compounds gives the oil a desirable bouquet reminiscent of fresh orange, while the absence of certain high-boiling compounds eliminates a heavy flavor note found in cold-pressed oils. Detailed analysis of essence oil from overripe oranges shows that relatively high boiling compounds are present and thus do contribute to the aroma of this essence oil. The 14 new compounds identified include the following sesquiterpene compounds: the hydrocarbons epi-α-selinene and nootkatene; the alcohols, elemol and intermedeol [the latter was reported by Sulser et al. (1971), as a possible precursor of nootkatone which plays an important role in grapefruit flavor (MacLeod and Buigues, 1964)]; the ketone, nootkatone; and the aldehydes, α- and β-sinensal. The sinensals are potent flavor compounds with a reported odor threshold of 308 ppb (Ahmed et al., 1978). The remaining new compounds are dodecanal, 1,8-*p*-menthadien-9-yl acetate, octyl acetate, terpene alcohols, 1,8-*p*-menthadien-9-ol, nerol, and geraniol, and *trans*-linalool oxide. Many of these compounds have unique aromas and, thus, could contribute to the difference in aroma and, probably, flavor between the essence oils from ripe and overripe fruit.

Table II summarizes the results from aroma preference tests, as determined by an expert panel, comparing essence oils from oranges picked during the normal harvesting period and from those considered overripe. There is a significant preference (99% confidence level) for essence oil from oranges harvested during the regular season. The aroma quality of essence oil from overripe fruit seems to have suffered from a combination of decreasing volatile compounds responsible for a fresh fruit bouquet and more high-boiling compounds which give the oil a heavier aroma note. The same panel (Table II) showed that the essence oil from overripe fruit could be blended in about a 1:9 ratio with good quality essence oil and no significant aroma preference for the control could be detected.

Table III shows the quantitative composition of whole essence oil from Valencia oranges. Essence oil contains a higher percentage of valencene than does cold-pressed peel oil and thus is considered an excellent commercial source of valencene which can be converted to nootkatone, an important flavor component of grapefruit. Quantitative analytical comparison of overripe essence oil with commercial essence oil in this limited study shows a significant increase in valencene in oil from overripe fruit. The increase from ~0.7% to ~1.5% appears to make essence oil from overripe oranges a better source than that previ-

Table III. Estimated[a] Quantities of the Major Compounds in Whole Essence Oil from Valencia Oranges

compound	% area under curve: overripe ess. oil	control ess. oil
α-pinene	0.34	0.46
myrcene	1.98	2.76
limonene	92.6	93.7
linalool	0.71	0.74
trans-2,8-*p*-menthadien-1-ol	0.14	0.04
cis-2,8-*p*-menthadien-1-ol	0.36	0.16
decanal	0.48	0.32
trans-carveol	0.25	0.06
carvone	0.12	0.13
perillaldehyde	0.11	0.04
dodecanal	0.08	0.04
β-elemene	0.09	0.07
β-caryophyllene	0.05	0.01
valencene	1.49	0.70
epi-α-selinene	0.08	0.04

[a] Estimates are based on gas chromatographic data.

ously believed to be the best source for obtaining valencene.

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Stereochemistry of Aflatoxicol B

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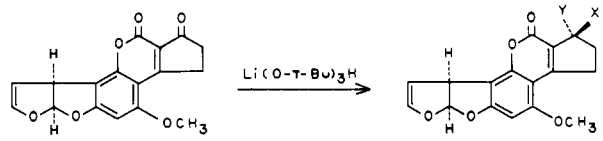
The relative stereochemistry of aflatoxicol B, one of the two diastereoisomers produced upon reduction of aflatoxin B_1 with lithium tris(*tert*-butoxy)aluminum hydride, has been determined by single-crystal X-ray diffraction analysis and corresponds to structure 2. Aflatoxicol B crystallized in the orthorhombic space group $P2_12_12_1$ with a = 16.463 (2), b = 19.178 (2), and c = 4.342 (1) Å. The structure was solved by direct methods and refined by full-matrix, least-squares methods to R = 0.04.

Aflatoxicols A and B (Cole et al., 1972; Detroy and Hesseltine, 1970) (aflatoxin R_0) are biological metabolites of aflatoxin B_1 and have been shown to produce many of the toxic effects found in aflatoxin B_1. Although less toxic than aflatoxin B_1, the aflatoxicols are carcinogens to trout (Schoenhard et al., 1974) and are active in the duckling bioassay (Detroy and Hesseltine, 1970) and in a microsomal mediated bacterial assay. Of the two diastereoisomers, aflatoxicol A (silica gel G-HR, chloroform–acetone 93:7 v/v, R_f 0.30) has been shown to be more toxic than aflatoxicol B (R_f 0.26).

Although the gross chemical structures of the aflatoxicols have been known for some time, the stereochemical detail of their structures has not been reported. Because the two diastereoisomers differ in toxicity, it was of interest to determine the stereochemistry of these isomers by single-crystal X-ray diffraction analysis.

RESULTS AND DISCUSSION

The reduction of aflatoxin B_1 (1) with lithium tris(*tert*-butoxy)aluminum hydride produce the two diastereoisomers 2 and 3 (Pawlowski et al., 1977). Separation by TLC



1

2: X=H, Y=OH
3: X=OH, Y=H

and recrystallization from chloroform–hexane produced suitable crystals of aflatoxicol B (R_f 0.26) for X-ray structure determination. The crystals were determined to be orthorhombic and belong to space group $P2_12_12_1$. Intensity measurements of diffraction maxima provided 813 unique nonzero reflections used in the structural analysis. The structure was determined by direct methods and refined by full-matrix least-squares procedures to an R = 0.040 (see Supplementary Material Available paragraph). Carbon and oxygen atoms were refined anisotropically and hydrogen atoms isotropically.

Tables I and II contain bond lengths and angles calculated from the final positional coordinates and Figure 1 shows a stick diagram and numbering scheme for the structure. The numbering scheme follows that in the previously reported structure of aflatoxin B_1 (van Soest and Peerdeman, 1970a,b,c). Bond lengths and angles are

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