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Compounds New to Essential Orange Oil from Fruit Treated with Abscission Chemicals

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The compositions of volatile constituents in essential oils from control and chemically treated Hamlin, Pineapple, and Valencia oranges were compared. The chemicals were abscission agents used to loosen fruit for mechanical harvesting. Six phenolic ethers—eugenol, methyleugenol, *cis*-methylisoeugenol, *trans*-methylisoeugenol, elemicin, and isoelemicin—were isolated and identified as citrus constituents for the first time, but only from the chemically treated fruit. Threshold levels in orange juice were determined for four of these ethers. The concentrations of all six compounds in the essential oil and processed juice from oranges were estimated.

The importance of mechanically harvesting citrus fruit has been accentuated in the last 10 years by over a 70% increase in cost of product production, a 25% increase in citrus available for processing, and the difficulty in retaining a stable work force. All promising mechanical harvesting systems include the application of abscission-inducing chemicals to loosen the fruit prior to harvesting.

Cooper et al. (1969) were first to report the use of cycloheximide (Acti Aid) as an effective abscission agent for citrus. However, cycloheximide was not suitable for late season (Valencia) oranges because it induced excessive droppage of the young fruit that were due to ripen the next season (Cooper and Henry, 1973). This problem was overcome by the use of two newer abscission agents: 5-chloro-3-methyl-4-nitro-1*H*-pyrozol (Release) reported by Wilson (1973) and glyoxal diamine (Pik-Off) reported by Wilcox et al. (1974). All three agents damage the peel, and this causes the fruit to release ethylene which promotes abscission. These chemicals have been shown to affect the chemical composition of cold-pressed orange oil and, thus, the flavor quality of processed juice and essential oil (Moshonas et al., 1976; Moshonas and Shaw, 1977). The change in chemical composition of the chemically treated oil could decrease the value of resulting citrus products if flavor changes are pronounced and detrimental. There is, therefore, a clear need to determine and evaluate the compositional changes brought about by the use of abscission-inducing agents. To date there has not been a systematic analysis to isolate and identify specific compounds that may be responsible for the compositional and flavor changes reported in previous studies.

We undertook to analyze essential oils from oranges treated with abscission agents, and now report six compounds not previously reported as citrus oil constituents.

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EXPERIMENTAL SECTION

Sample Preparation. Each pair of samples, which included untreated control oranges and oranges treated with one of the listed abscission agents, was harvested from adjacent trees on similar rootstock. The trees had been sprayed, fertilized, and irrigated the same way. Samples were prepared both from oranges that had just reached legal maturity and from well-matured fruit of early- (Hamlin), mid- (Pineapple) and late-season (Valencia) oranges. Oranges were thoroughly washed and then processed with a commercial 5-cup FMC In-Line Extractor. The extracted crude oil emulsions were put through 0.20-in. screen openings and 30- and 60-mesh shaker screens for removal of residual solid materials. Each emulsion was then placed into a holding tank for 4 h, after which most of the water layer was removed. The oil rich emulsion was then separated in a continuous-type centrifuge operated at a relative centrifugal force of about 22 000*g*, which yielded the cold-pressed orange peel oil. The oils were stored at 0 °C until they were analyzed.

Separation Procedure. Each control and experimental orange oil sample (300 mL) was placed in a rotary evaporator and distilled at 36 °C at a pressure of 1–2 mm until most of the terpene hydrocarbons (99% limonene) were removed. A 4-g sample of the 13.3 g of residue was separated into three fractions on a 1 in. × 15 in., 9 °C water-jacketed column containing 100–200 mesh Florisil deactivated with 6% water (Lund and Coleman, 1977).

Table I. Flavor Thresholds and Estimated Amounts (in ppb) of New Compounds Present in Juice from Fruit Treated with Abscission Chemicals

compd	flavor threshold	amount present
eugenol	22	21
methyleugenol	1250	42
elemicin	22000	10
trans-methylisoeugenol	35000	21
cis-methylisoeugenol	ND[a]	4.2
trans-isoelemicin	ND	31.5

[a] Not determined.

The fractions were eluted successively with 300 mL of distilled hexane to remove the hydrocarbons, 300 mL of a 1:1 mixture of hexane and ethyl ether to remove a carbonyl-rich fraction and 300 mL of absolute ethanol to remove the alcohols. Removal of the solvents by vacuum distillation yielded 0.9 g of hydrocarbons, 2.3 g of carbonyl-rich fraction, and 0.6 g of alcohols. The distillate and each column fraction were further separated into individual compounds by gas chromatography (GLC). Fractions were analyzed on a Perkin-Elmer Model 900 gas chromatograph, equipped with a thermal conductivity detector. Constituents were separated on a 0.10 in. i.d. × 20 ft column packed with 10% UCW-98 on 60–80 mesh Gas-Chrom P. Oven temperature was programmed from 90 to 110 °C at 1 °C/min, then to 230 °C at 4 °C/min; and helium flow was 30 mL/min. Injector port temperature was 275 °C and detector temperature was 290 °C. Individual compounds were collected as they were eluted from the GLC and positively identified by comparison of their infrared spectra, mass spectra, and retention times with those of authentic samples.

Quantitative data in Table I were determined on a typical oil sample, which was obtained from Pineapple oranges sprayed with 10 ppm cycloheximide and picked 6 days later. The carbonyl-rich fraction, which contained the phenolic derivatives listed in Table I, was used for the quantitative analysis by GLC. A Hewlett-Packard Model 3380A computing integrator coupled to the gas chromatograph measured GLC peak areas. Amounts of each compound present in juice, as listed in Table I, were calculated on the basis that the juice contained 0.0175% oil.

Syntheses of Elemicin and Isoelemicin. Elemicin was synthesized by the procedure of Shulgin (1965) and purified by distillation [bp 91–92 °C (0.050 mm)]. The distillate was shown to be only 91% pure by GLC and was further purified by chromatographing a 3-g portion placed on 90 g of Silica gel and eluting with 100 mL of hexane. The eluate was concentrated and distilled in a micro bulk apparatus at 88–103 °C oil bath temperature and 0.05 mm pressure to afford 1.1 g of distillate that was >97% pure by GLC, UV λ max 215 nm (ϵ 17 300), 271 nm (ϵ 840). A sample of elemicin isolated by GLC from orange oil treated with abscission chemical showed UV λ max 213 nm (ϵ 13 100), 270 nm shldr/(ϵ 1430).

Isoelemicin was prepared by the procedure of Shulgin (1965) and the yellow oily residue was separated by GLC into its cis and (mostly) trans isomers. The isomers were identified by comparison of their infrared spectra with those of authentic samples (Shulgin, 1965).

Flavor Evaluations. Taste thresholds of new compounds were determined by the method described by Harrison and Elder (1950). Each taster was presented a series of paired single-strength orange juice samples, randomly arranged. Each pair consisted of one dilution of the test compound and an untreated sample. Threshold level was that concentration correctly identified by 75% of the panel.

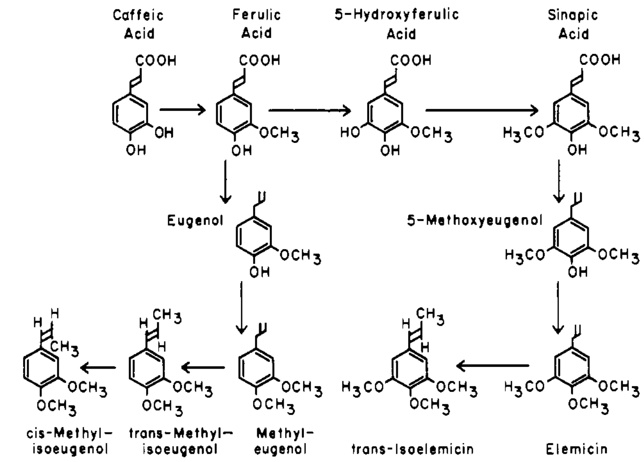

Figure 1. Possible pathways for formation of phenolic ethers in orange oils.

RESULTS AND DISCUSSION

All abscission agents that have been used successfully to loosen oranges for mechanical harvesting are of the rind-injuring type. Our analysis of 19 experimental oils from oranges treated with these agents showed they affect a particular metabolic pathway within the orange, regardless of cultivar, rootstock, maturity, fertilizer or irrigation practices, causing the production of the same group of phenolic ethers. The analytical procedures used to analyze control and experimental orange oil samples were identical. None of these phenolic ethers were found in any of the 19 corresponding control oils from untreated fruit. Oils from oranges treated with 2-chloroethylphosphonic acid (ethephon), which does not injure the rind and which has limited use as a citrus loosening aid, were also analyzed and found not to contain the phenolic ethers. The six ethers were eugenol, *cis*-methylisoeugenol, *trans*-methylisoeugenol, methyleugenol, elemicin, and isoelemicin; and this is the first report of their presence in citrus fruit. Fifty-two other compounds were isolated in control and experimental oils and they have been previously reported as orange components (Shaw, 1977). Figure 1 shows the structure of each ether in a scheme of possible pathways by which they might be formed. The proposed pathways are based on findings of Tressl and Drawert (1973). They reported that banana discs converted [1-^{14}C]caffeic acid into labeled eugenol, methyleugenol, and elemicin. The phenolic acids and enzymes involved in these reactions have been found in citrus fruit (Raymond and Maier, 1977).

Table I shows flavor thresholds of four of the phenolic ethers in single-strength orange juice. Eugenol was the most flavor potent with a flavor threshold of 22 ppb in orange juice, followed by methyleugenol 1.25 ppm, elemicin 22 ppm, and *trans*-methylisoeugenol 35 ppm. The threshold levels fall in the range that an individual would consume from other foods. Arctander (1969) reported that eugenol, at 10 to over 100 ppm, is used to give a clove-like note to combination spice flavors and to modify complex flavors such as those of nuts, mint, and various fruits; that *trans*-methylisoeugenol, at 5 to 100 ppm is used as a flavor component of spice blends, vanilla imitations and chocolate bases; and that methyleugenol, at 5 to 15 ppm is used as a flavor component of spice blends and baked goods. The concentration of elemicin due to the nutmeg was determined to be above 22 ppb in a single portion of a pudding. Flavor thresholds for isoelemicin and *cis*-methylisoeugenol were not obtained because, although they are found in food products, we could not determine their concentrations in foods and thus could not be certain of a safe level for human consumption. To study the possible additive flavor contribution of these ethers, we compared control orange juice with juice to which we had added eugenol, methyleugenol, and *trans*-methylisoeugenol at half their threshold levels and compared to a control sample. The taste panel distinguished the test flavor sample at the 99.9% confidence level. Concentration of each phenolic ether in experimental orange oil was estimated from GLC peak areas. These oils were estimated to contain 240 ppm methyleugenol, 180 ppm isoelemicin, 120 ppm eugenol, 120 ppm *trans*-methylisoeugenol, 60 ppm elemicin, and 24 ppm *cis*-methylisoeugenol.

Although none of the ethers was present at the flavor threshold level, their flavor effects are additive and the combined effect was apparently sufficient to account for off-flavor of processed single-strength orange juice reported by Moshonas et al. (1976). One compound in particular, eugenol, was present in orange juice at approximately its flavor threshold.

Increased amounts of phenolic ethers were present when oranges were treated with higher concentrations of abscission agents. In one comparison, oil from Pineapple oranges treated with 20 ppm cycloheximide had higher levels of these ethers than oils from comparable fruit treated with 10 ppm cycloheximide, as indicated by the relative GLC peak areas; however, the absolute quantities of these compounds present were not determined.

In practice, several factors tend to minimize any effect on flavor of processed citrus products that the abscission agent may produce. By normal processing practices, juice from fruit treated with abscission agents would be mixed with much larger volumes of juice from untreated fruit so that flavor effects would be reduced by dilution. Also, preparation of concentrated juice tends to reduce, but not eliminate, flavor effects (Moshonas et al., 1976). As mechanical harvesting of citrus becomes more widely adopted, these factors that minimize flavor effects will diminish; thus, it will be increasingly important to carefully control the concentration of abscission agents and to minimize the time required for fruit loosening after spraying. A new generation of abscission agents, composed of mixtures of the chemicals reported in this study, are effective at lower concentrations than those of the individual agents. Even though preliminary qualitative results show that the mixtures also induce formation of the phenolic ethers, hopefully the levels are low enough that flavor effects are minimal.

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Volatile Components of Corn Silk (*Zea mays* L.): Possible *Heliothis zea* (Boddie) Attractants

Robert A. Flath,* Ralph R. Forrey, Janie O. John, and Bock G. Chan

Volatile components of corn silk were concentrated by vacuum co-distillation with water, followed by ether extraction of the distillate. The concentrate was examined by large bore capillary gas chromatography–mass spectrometry, and 63 compounds were identified. Alcohols comprise the major portion of the concentrate, both in number and in quantity, with 2-heptanol being the major constituent. A highly odorous compound, geosmin, was found among the volatiles. Preliminary attractancy testing with gravid *Heliothis zea* (Boddie) moths yielded inconclusive results.

The manner in which predator insects locate host plants is still subject to controversy (Kennedy, 1977), but it is generally held that some sort of insect response to volatiles emitted by the host plant is involved in causing the insect to locate the host. Other contributing factors suggested have included differences in infrared radiation between the host plant and its surroundings, plant color, and perhaps shape or texture of plant parts. In dealing with plant volatiles as possible predator attractants, it would be desirable to determine what volatiles are present in the plant, whether the total volatiles vary in composition with the segment of the plant inspected, and whether these volatiles have any effect on the insect predator's behavior.

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