on the plate. The results show that the purple spot with $R_f$ 0.49 is N-nitroso-p-methoxy-N-dodecylaniline, which on irradiation undergoes denitrosation to the parent amine and dehydronitrosation to the imine, with the latter undergoing further cleavage to anisidine and dodecanal. When solutions 0-4 (Table VII) were analyzed by TLC on an alumina plate as above, a pink spot now known to be o-nitro-p-methoxy-N-dodecylaniline was clearly visible and increased in intensity on going from sample 0 to sample 3. Irradiation of the plate for 15 min under UV light made three more spots visible. The first ($R_f$ 0.61) was purple and coincident with p-methoxy-N-dodecylaniline. This spot did not appear until sample 3. The purple spot ($R_f$ 0.49), identified as described above as N-nitroso-p-methoxy-N-dodecylaniline, was relatively constant in samples 0-2 but decreased dramatically in sample 3 and was absent in sample 4. The orange spot ($R_f$ 0.19), representing as proven above the N-nitroso-C-nitroso compound, decreased steadily through the series and was practically absent in sample 4. It would thus appear that the crude nitrosated product from p-methoxy-N-dodecylaniline on heating to about 200 °C decomposes extensively to give the parent amine and o-nitro-p-methoxy-N-dodecylaniline, presumably via rearrangement of the N-nitroso to the o-C-nitroso compound followed by air oxidation. In this respect, the inhibitory effect of p-methoxy-N-dodecylaniline in NA formation in bacon is very reminiscent, mechanistically speaking, of that of ethoxyquin described in an earlier paper from this laboratory (Bharucha et al., 1985).

In summary, p-alkoxyanilines, both primary and secondary, are excellent antinitrosamine agents in bacon, with the latter more efficient than the former. Ortho-, meta-, and phenoxy-substituted anilines also inhibit NA formation, but somewhat less efficiently. The mechanism of inhibition appears similar to that of ethoxyquin, viz. initial N-nitrosation followed by rearrangement and oxidation to the o-nitro compound in the case of secondary amines.

**Registry No.** $p$-MeOC$_6$H$_4$NH(CH$_2$)$_{11}$Me, 54574-77-5; $p$-EtOC$_6$H$_4$NH(CH$_2$)$_{11}$Me, 65570-08-3; $p$-Me(CH$_2$)$_{11}$OC$_6$H$_4$NH-(CH$_2$)$_{11}$Me, 65570-10-7; $p$-MeOC$_6$H$_4$NHEt, 104-48-3; $p$-EtOC$_6$H$_4$NHEt, 65570-13-0; $p$-MeOC$_6$H$_4$NHMe, 5961-59-1; $p$-MeOC$_6$H$_4$NHBu, 61829-43-4; $p$-MeOC$_6$H$_4$NH(CH$_2$)$_5$Me, 16664-54-3; $p$-EtOC$_6$H$_4$NHMe, 3154-18-5; $p$-EtOC$_6$H$_4$NHBu, 15498-39-2; $p$-EtOC$_6$H$_4$NH(CH$_2$)$_5$Me, 16663-90-4; $p$-MeOC$_6$H$_4$NHCH$_2$C(Me)$_3$, 65570-14-1; $p$-$sec$-BuOC$_6$H$_4$NH(CH$_2$)$_{11}$Me, 65570-15-2; $p$-MeOC$_6$H$_4$NHBu-$t$, 15408-62-5; $p$-MeOC$_6$H$_4$NHCH$_2$Ph, 17377-95-6; $p$-MeOC$_6$H$_4$NHCH$_2$CH(Ph)Et, 65570-16-3; $o,p$-(MeO)$_2$C$_6$H$_3$NH(CH$_2$)$_{11}$Me, 65570-17-4; $m$-MeOC$_6$H$_4$NH-(CH$_2$)$_{11}$Me, 65570-18-5; PhNH(CH$_2$)$_{11}$Me, 3007-74-7; $p$-PhOC$_6$H$_4$NH(CH$_2$)$_5$Me, 65570-11-8; $o$-PhOC$_6$H$_4$NH(CH$_2$)$_5$Me, 65570-19-6; $o$-MeOC$_6$H$_4$NH(CH$_2$)$_5$Me, 65570-21-0; $o$-BuOC$_6$H$_4$NH(CH$_2$)$_{11}$Me, 65606-63-5; $o$-MeOC$_6$H$_4$NHEt, 15258-43-2; $o$-Me(CH$_2$)$_{11}$OC$_6$H$_4$NH(CH$_2$)$_{11}$Me, 103439-76-5; $o$-MeOC$_6$H$_4$NHBu, 65570-20-9; $o$-MeOC$_6$H$_4$NH(CH$_2$)$_{11}$Me, 65570-22-1; $p$-PrOC$_6$H$_4$NH$_2$, 4469-80-1; $p$-MeOC$_6$H$_4$NH$_2$, 104-94-9; $p$-EtOC$_6$H$_4$NH$_2$, 156-43-4; $p$-BuOC$_6$H$_4$NH$_2$, 4344-55-2; $p$-Me-(CH$_2$)$_5$OC$_6$H$_4$NH$_2$, 39905-57-2; $p$-Me(CH$_2$)$_6$OC$_6$H$_4$NH$_2$, 39905-44-7; $p$-Me(CH$_2$)$_{11}$OC$_6$H$_4$NH$_2$, 65039-19-2; $p$-PhOC$_6$H$_4$NH$_2$, 139-59-3; $o$-PhOC$_6$H$_4$NH$_2$, 2688-84-8; $o$-MeOC$_6$H$_4$NH$_2$, 90-04-0; $o$-EtOC$_6$H$_4$NH$_2$, 94-70-2; $o$-BuOC$_6$H$_4$NH$_2$, 4469-81-2; $p$-$sec$-BuOC$_6$H$_4$NH$_2$, 59002-72-1; $m$-MeOC$_6$H$_4$NH$_2$, 536-90-3; $o,p$-(MeO)$_2$C$_6$H$_3$NH, 2735-04-8; p-methoxy-2-methylaniline, 102-50-1; p-methoxy-o-nitro-N-dodecylaniline, 103439-77-6; ethoxyquin, 91-53-2.

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# Valencia Orange Leaf Oil Composition

Manuel G. Moshonas* and Philip E. Shaw

Leaf oils both from Valencia orange trees treated with the abscission agent 5-chloro-3-methyl-4-nitro-1H-pyrazole (Release) and from untreated trees were analyzed, and 36 compounds were identified; seven of them are being reported for the first time as components of orange leaf oil. 4-Vinylguaiacol was found for the first time as a natural citrus constituent. It had been reported previously as a degradation product and artifact in stored canned single-strength orange juice. Other newly found orange leaf oil components were α- and β-sinensal, geranyl acetate, m-cymene, p-cymene, and cis-p-2-menthen-1-ol.

Abscission-inducing chemicals are used to loosen citrus fruit prior to mechanical harvesting. Moshonas et al. (1976, 1977) showed that these chemicals affect the quality of processed orange juice and cold-pressed peel essential oil. Six phenolic ether compounds isolated from chemically treated fruit were identified and shown to be compounds that do not normally occur in citrus but are formed by a change in metabolic pathways brought about by the abscission agents (Moshonas and Shaw, 1978). Because the effect of these phenolic ethers was characterized as contributing an "overripe" flavor, a study was made that showed that these compounds were not formed when fruit were allowed to stay on the tree until they were extremely overripe (Moshonas and Shaw, 1979).

Since the leaves are also sprayed and are loosened by the abscission agents used to loosen fruit, the current study was made to determine whether phenolic ethers or other

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Valencia Orange Leaf Oil Composition

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**Table I. Orange Leaf Oil Components**

| hydrocarbons | esters | alcohols |
|---|---|---|
| α-pinene | citronellyl acetate | p-cymen-8-ol[a] |
| sabinene | neryl acetate | linalool |
| δ³-carene | geranyl acetate[a] | terpinen-4-ol |
| limonene | | cis-p-2-menthen-1-ol[a] |
| ocimene | aldehydes | cis-3-hexen-1-ol |
| p-cymene | α-sinensal[a] | isopulegol |
| m-cymene[a] | β-sinensal[a] | nerol |
| α-thujene | neral | geraniol |
| myrcene | geranial | α-terpineol |
| γ-terpinene | citronellal | trans-2-hexen-1-ol |
| terpinolene | | 1-hexanol |
| β-caryophyllene | ketones | citronellol |
| humulene | 2-methyl-hept- | |
| β-elemene | 2-en-6-one | |
| | phenols | |
| | 4-vinylguaiacol[a,b] | |

[a] Compounds identified for the first time from orange tree leaves. [b] Compounds identified for the first time as citrus constituents.

new constituents are formed by leaves in trees exposed to abscission agents, as well as in leaves from untreated trees. This investigation also sought to extend the knowledge of the chemistry of essential oils of citrus leaves.

## EXPERIMENTAL SECTION

Mature Valencia orange leaves [*Citrus sinensis* (L.) Osbeck cv. Valencia] were picked from tees that had been sprayed 7 days earlier with 350 ppm of 5-chloro-3-methyl-4-nitro-1*H*-pyrazole (Release), as described by Clark and Wilson (1975). These leaves and leaves from trees that were untreated were thoroughly washed and allowed to air-dry. The dried leaves (2800 g) were steam distilled at atmospheric pressure the day after they were harvested. The volatile product was condensed with chilled water (9 °C), and the oil (4.8 g) was separated by centrifugation at 1600g at 10 °C.

**Separation Procedure.** Valencia orange leaf oil (2.4 g) was placed in a chilled water-jacketed column (1 × 15 in.) containing 60/80-mesh Florisil (Fisher Scientific Co.) deactivated with 6% water. The column was washed with 300 mL of hexane to elute hydrocarbons, 300 mL of a 2:1 hexane–ethyl ether mixture to elute carbonyl-containing compounds, and 300 mL of absolute ethanol to elute the remaining compounds. Further separation of individual compounds was accomplished by gas chromatography (GC) using a Perkin-Elmer Model 900 gas chromatograph equipped with a thermal conductivity detector and a 0.10-in. i.d. × 20-ft column packed either with 10% Carbowax 20M or with 10% UCW-98 on 60/80-mesh Gas-Chrom P. The injection port and the detector temperatures were 275 °C for all runs. 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 GC and were positively identified by comparison of their infrared spectra and retention times with those of authentic samples. The GC curves for the centrifuged oil samples were determined on the UCW-98 column as described above except the oven temperature was programmed at a rate of 1.5 °C/min.

## RESULTS AND DISCUSSION

Valencia orange leaves from trees treated with an abscission chemical and leaves from untreated trees were steam distilled, and the leaf oils thus obtained were analyzed for compositional differences. Table I lists the 36 compounds isolated and identified from these leaf oils. A gas chromatographic profile of centrifuged leaf oils from treated and untreated trees showed no qualitative or

marked quantitative differences (based on peak heights) in composition. Detailed analysis of column chromatographic fractions isolated from leaf oils prepared from treated and untreated leaves also showed no compositional differences. These results are in contrast to those found earlier with peel essential oil from Valencia orange fruit (Moshonas and Shaw, 1978) where six phenolic ethers not found previously in citrus were identified in the peel oil from fruit treated with abscission chemicals. If present in leaves, these ethers should have been detected since they would steam distill (McHall et al., 1977). The phenolic ethers could have been synthesized in the leaves and transported to the fruit. However, that source for their presence in the fruit seems unlikely, since they were not identified in the leaf oils.

In this study, seven components were found as orange leaf constituents for the first time (Table I). They include α- and β-sinensal, two isomeric components of orange peel oil in which the β isomer predominates (Wilson and Shaw, 1984); β-sinensal is the predominant isomer in leaf oil as well. p-Cymen-8-ol (α,α,4-trimethylbenzyl alcohol) had been identified earlier in several citrus peel essential oils (Shaw, 1977) but not in citrus leaf oils. It is a possible source, through dehydration, of α,4-dimethylstyrene. This hydrocarbon was found in several citrus leaf oils, and its quantity varied significantly depending on the cultivar (Kamiyama, 1970).

Other components not reported earlier as constituents of orange leaf oil were cis-p-2-menthen-1-ol, geranyl acetate, m-cymene, and 4-vinylguaiacol. cis-p-2-Menthen-1-ol was reported in lemon and lime aqueous essences (Moshonas and Shaw, 1972), and geranyl acetate and m-cymene have been reported as volatile citrus components (Van-Straten and de Vrijier, 1973). 4-Vinylguaiacol has been found in the orange only as a degradation product formed during storage of canned single-strength juice (Tatum et al., 1975). It contributed an overripe flavor note to orange juice. However, it was not found in a flavor fraction (essence oil) from overripe oranges that contained the water-soluble and oil-soluble volatile constituents (Moshonas and Shaw, 1979).

In conclusion, analyses of leaf oils from trees sprayed with an abscission agent showed no qualitative differences in composition when compared to leaf oils from untreated trees. Several compounds not reported earlier as orange leaf oil components were identified in both leaf oil samples.

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# Identification of Germacrene D in Walnut and Fig Leaf Volatiles

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By capillary GLC–MS and packed-column GLC with batch IR, germacrene D was identified as a major volatile component of both walnut (ca. 0.5 ppm) and fig (ca. 0.4 ppm) tree leaves. Other major volatiles also identified include caryophyllene, (E)-β-ocimene, β-pinene, and limonene in walnut leaves and (Z)-3-hexenol, (Z)-3-hexenyl acetate, methyl salicylate, and β-cyclocitral in fig leaves.

## INTRODUCTION

Some of us have been carrying out studies to identify the volatile constituents of leaves and other parts of important crops (cf. Buttery and Ling, 1984). Such volatile compounds might be involved in the attraction of some insect pests to these crops. Two important tree crops in California are walnuts and figs. The walnut husk fly (Rhagoletis completa Cresson) is a pest of walnuts. Fig pests include various Nitidulids and Drosophila species. Although the insects attack the fruits, the major volatiles of the plant are probably given off by the considerably greater quantity (and area) of leaves. These may initially attract the insects to the tree.

Some previous studies have been reported on the nonvolatile constituents of fig leaves (e.g., Innocenti et al., 1982), but none were found on the volatiles of fig leaves. Some identification of volatile components of walnut leaves had been reported (Popescu and Ciupe, 1972; Kolesnikova, 1980) from thin-layer chromatography and evidence based on $R_f$ values. These previous workers had found such evidence for the presence of juglone, α- and β-pinene, limonene, δ-3-carene, 1,8-cineole, bornyl acetate, α-terpineol, and cadinenes.

As identification using thin-layer $R_f$ values alone is of doubtful value, we felt that it was necessary to reinvestigate the volatiles of the leaves by the more certain method of the capillary GLC–mass spectrometry combination and, where possible, infrared absorption spectra.

## EXPERIMENTAL SECTION

**Materials.** Walnut tree leaves were obtained from the English walnut (Juglans regia) growing in El Cerrito, CA, in the spring and summer of 1984. Fig leaves were obtained from the black Mission fig (Ficus caprica) growing in El Cerrito, CA, during the spring and summer of 1983. The intact leaves (kept at 20–25 °C) were used within 4 h after picking. Care was taken that the leaves were not crushed or otherwise damaged.

**Isolation of Volatile Oil from Walnut Leaves.** The method used was essentially the same as described previously by us for other crops such as corn leaves (Buttery and Ling, 1984). The intact walnut leaves (500 g) were placed in a 12-L flask. A Tenax trap (14-cm length × 2.2 cm diameter; 10 g of Tenax) was attached to the neck of the flask. Air drawn from outside the laboratory (and purified by passage through activated charcoal) was led into the flask through a Teflon tube and out through the Tenax trap. The flow of air was 1 L/min and was continued for 24 h. The trapped volatiles were eluted from the trap with freshly distilled diethyl ether (containing ca. 0.001% of ethyl antioxidant 330). The ether extract was then concentrated to a small volume (20 µL) on a warm-water bath and low-hold-up micro Vigreux type distillation columns.

The particular type of trapping method used was developed on the basis of theoretical calculations involving the volatilities of sesquiterpene and other common plant volatile compounds for the largely aqueous tissue media and for the surface wax layer. Experiments carried out with standard sesquiterpenes, aliphatic aldehydes, and alcohols showed good recovery of all compounds with no noticeable oxidation despite the large volume of air used. The Tenax traps were reactivated in the normal way by heating at 220 °C in a nitrogen stream. A trace of the nonvolatile ethyl antioxidant 330 is left on the Tenax from the elution process. No background was found in blank tests, and the trace antioxidant probably acts as an extra protection to the plant volatiles.

**Isolation from Fig Leaves Using Tenax Trapping.** This was carried out by exactly the same procedure as used with walnut leaves.

**Isolation from Fig Leaves Using Vacuum Steam Distillation.** The intact fig leaves (1 kg) were placed in a 12-L flask and covered with water (6 L). A Likens-Nickerson type steam distillation continuous-extraction head (cf. Nickerson and Likens, 1966) was connected to the neck of the flask. A 250-mL flask containing ca. 120 mL of purified hexane (with a trace of ethyl antioxidant 330) was attached to the solvent arm of the head. Vacuum

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