brix-acid ratio be maintained, it is suggested that both be tested for δ ^{13}C. For example, in Table I are included results of SIRA for citric acid and sugars in lemon juice adulterated to different levels with corn sugar fermentation-derived citric acid and HFCS. The higher the degree of adulteration, the less negative are the δ ^{13}C values.

δ ^{13}C values of citric acid from lemon juices may be especially useful for imported samples. Citric acid from an Argentinean sample of allegedly pure lemon juice gave a value of -17.7‰. This suggests adulteration with citric acid from a C_4 fermentation source.

In summary, SIRA should be included in the series of analytical strategies aimed at detecting lemon juice adulteration. C_4 plant derived citric acid can be detected, and in some cases, possibly so can paraffin derived citric acid. Sugars from corn or cane syrups also can be detected on the basis of δ ^{13}C values of this fraction.

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Registry No. Citric acid, 77-92-9; carbon-12, 7440-44-0; carbon-13, 14762-74-4.

LITERATURE CITED

Doner, L. W.; Bills, D. D. *J. Assoc. Off. Anal. Chem.* **1982**, *65*, 608.
Krueger, H. W. *Am. Lab.* (Fairfield, Conn.) **1984**, *90*.
Nissenbaum, A.; Lifshitz, A.; Stepak, Y. *Lebensm. Wiss. Technol.* **1974**, *7*, 152.
Petrus, D. R.; Vandercook, C. E. In "Citrus Nutrition and Quality"; Nagy, S., Attaway, J. A., Eds.; American Chemical Society: Washington, DC, 1980; Chapter 17.
Silverman, S. R. *J. Am. Oil Chem. Soc.* **1967**, *44*, 691.
Swisher, H. E.; Swisher, L. H. In "Fruit & Vegetable Juice Processing Technology"; Nelson, P. E., Tressler, D. K., Eds.; AVI Publishing Co.: Westport, CT, 1980; Chapter 4.
Winkler, F. J.; Schmidt, H. L. *Z. Lebensm.—Unters. Forsch.* **1980**, *171*, 85.

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Volatile Components of Corn Roots: Possible Insect Attractants

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The volatiles of corn roots have been studied by using Tenax adsorbent trapping followed by capillary GLC–MS analysis. Sesquiterpene hydrocarbons were the principal components of the low concentration (ca. 10 ppb) of volatiles found. The major sesquiterpene hydrocarbon could not be identified. Others that were identified included β-caryophyllene, longifolene, bazzanene (tentative), cyclosativene, and α-ylangene.

INTRODUCTION

Corn roots are attacked by a number of insects such as the corn root worm (*Diabrotica* species). It seems possible that these insects locate the corn roots by orienting toward some characteristic volatile odor compound associated with the roots. The authors had previously identified volatile compounds associated with the corn leaves (Buttery and Ling, 1984) with some preliminary observations on the corn roots. The present study was carried out to give a more complete identification of corn root volatiles.

EXPERIMENTAL SECTION

Materials. Corn roots were obtained from corn plants grown in two different areas during the summer of 1983 and 1984. These were Stylepak and Jubilee varieties grown on an experimental field at Berkeley and Bonanza variety grown on an experimental field at Davis, CA. The plants were pulled out of the ground and the roots cut from the stalk with a sharp knife. Roots from relatively young plants (ca. 60 cm high) and mature plants (ca. 2 m high) were used for separate studies.

Isolation of Volatiles. The method used was essentially the same as that described previously by the authors for corn leaves (Buttery and Ling, 1984). The roots (800 g) were cut into pieces (ca. 4 × 4 × 6 cm) and placed in a 12-L flask within about 2 h of harvesting. Most of the soil on the roots was dislodged in the process of cutting but was not washed off because of the possibility of removing volatiles. The Tenax trap consisted of a Pyrex tube packed with 10 g of Tenax (14 cm long × 2.2 cm diameter). Air drawn from outside the laboratory (purified passing through activated charcoal) was led into the flask through a Teflon tube and passed over the roots and out through the Tenax trap. The flow of air was 1 L per min and was continued for 24 h. The trapped volatiles were eluted from the trap with freshly distilled diethyl ether containing a trace (less than 0.001%) of Ethyl antioxidant 330. The extracts from two collections were combined and then concentrated to a small volume (5 μL) by using a warm water bath and low hold up fractional distillation columns.

Capillary Gas Liquid Chromatography–Mass Spectrometry Analysis (GLC–MS). The capillary GLC column used for the main part of the work was a 150 m long by 0.66 mm i.d. Pyrex capillary wall coated with Silicone OV-3. For some of the work a Pyrex capillary of the same dimensions but wall coated with Carbowax 20-M was used. The silicone capillary was temperature programmed from 20 to 170 °C at 1 °C per min and held at 170 °C for 2 more h. The carbowax capillary was held at 60 °C for the first 40 min and then programmed to 170 °C at 1 °C per min and held at 170 °C for 2 h. The column inlet pressure was 15 p.s.i. He. The column was coupled to the mass spectrometer with a Llewellyn-Littlejohn type single stage silicone rubber membrane molecular separator. The mass spectrometer was a modified Consolidated 21-620 cycloidal type instrument with 70 eV ionization volt-

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Table I. Volatile Compounds Identified in the Atmosphere above Corn Roots with Tenax Trapping

compound[a]	major mass spec. ions[b] (one each 14 mass units)	Kovats' GLC index[c]	rel %
aliphatic aldehydes			
hexanal	44, 56, 72, 82	810	2–5
2-methyl-2-pentenal	41, 69, 98, 55, 83	850	7–23
sesquiterpene hydrocarbons			
cyclosativene	105, 94, 41, 161, 119, 204	1380	1–9
α-Ylangene	105, 119, 161, 93, 41, 81	1385	0.5–7
longifolene	161, 94, 41, 107, 189, 135	1420	2–12
β-caryophyllene	41, 69, 93, 79, 133, 55	1430	5–19
unknown sesquiterpene[e]	93, 80, 136, 121, 107, 41	1500	10–28
bazzanene[d]	109, 67, 93, 41, 55, 81	1520	1–11
other compounds			
benzaldehyde	77, 105, 51, 39	1020	0–2
linalool	93, 71, 41, 55, 80, 121	1120	0–2

[a] Complete mass spectrum and Kovats' GLC index are consistent with that of authentic samples except for *e* and *d*. [b] The most intense ion each 14 mass units above *m/z* 34. Ions in descending order of intensity with molecular ion (if listed) in italic type. [c] Kovats' GLC index for the Pyrex Silicone OV-3 capillary column. [d] No authentic sample was available but mass spectrum and GLC retention data are consistent with published data. [e] Unable to identify with certainty.

age. Separate GLC–MS runs were made on 5 different batches of roots.

Authentic Samples. β-Caryophyllene and longifolene were obtained from commercial sources (K&K and Aldrich). α-Ylangene and α-humulene were obtained from hop oil. Cyclosativene was obtained from the cortical oleoresin of *Abies magnifica* (cf. Smedman et al., 1969). α-Chamigrene was obtained by isomerization of thujopsene according to the method of Daeniker et al. (1972). All compounds were repurified by GLC separation and their identities verified by spectral means (MS and or IR) and capillary GLC retention data.

RESULTS AND DISCUSSION

The amount of volatiles obtained from the roots by Tenax trapping was of the order of 10 parts of volatiles per billion parts of the roots (10 ppb). This was based on the weight of roots used, the volume of the volatile concentrate and the GLC peak areas (compared to standards). The compounds identified by GLC–MS in the volatile concentrate are listed in Table I. Separate GLC–MS studies were carried out on five different batches of corn roots obtained at different stages of plant growth and from two different varieties and two different growing localities. The range of relative concentrations found (based on GLC peak areas) are also listed in Table I. The major group of compounds found were sesquiterpene hydrocarbons. The green leaf type compounds such as (*Z*)-3-hexenol and (*Z*)-3-hexenyl acetate and related compounds (found in relatively large amounts in the leaves; Buttery and Ling, 1984) were almost completely absent from the roots. The only member of this group found in the roots was hexanal.

Sesquiterpenes. Of the sesquiterpene hydrocarbons both cyclosativene and α-ylangene, previously found in corn leaves, were found in much smaller relative amounts in the roots. The identity of cyclosativene (only tentatively identified in the corn leaves; Buttery and Ling, 1984) was confirmed by direct comparison with an authentic sample of cyclosativene isolated from the cortical oleoresin of *Abies magnifica* (cf. Smedman et al., 1969). The mass spectrum and GLC retention data of the unknown were consistent with that of the authentic sample.

The mass spectral and GLC data for the sesquiterpene identified as α-ylangene were quite consistent with that of an authentic sample of this compound and different from those of the closely similar geometric isomer α-copaene.

The major volatile component of the roots could not be identified except for the fact that it is a sesquiterpene hydrocarbon. Its mass spectrum is shown in Figure 1.

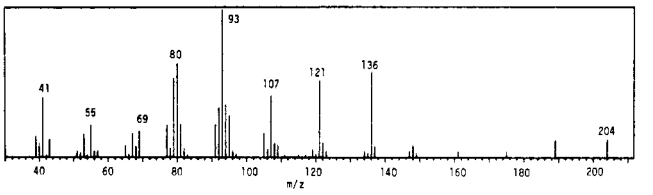

Figure 1. Mass spectrum of major (unidentified) sesquiterpene hydrocarbon of corn roots.

This mass spectrum is clearly that of a sesquiterpene hydrocarbon. The spectrum was compared with those of more than 70 known sesquiterpene hydrocarbons in the authors' library but was not consistent with any. The spectrum was most similar to that of α-chamigrene but also somewhat like that of γ-elemene and α-humulene (cf. Heller and Milne, 1978) but showed differences from all three. Its Kovats' GLC index (KI) was 1500 on the Silicone OV-3 Pyrex capillary column. This is consistent with the KI (1530) reported for α-chamigrene by Anderson et al. (1977) for Silicone SF96 stationary phase which is similar to the Silicone OV-3 used in the present work (making allowance for the absolute difference and using β-caryophyllene as an internal marker). The KI for the unknown on Carbowax 20-M (1730) was reasonably consistent with that of α-chamigrene (KI = 1790) reported by Anderson et al. (1977) again using β-caryophyllene as an internal marker. The KI for authentic γ-elemene on Silicone SF96 (KI = 1350) is quite different, eliminating it as a possibility. The KI of authentic α-humulene on Silicone SF96 (KI = 1470), however, is also close. An authentic sample of α-chamigrene was obtained following the procedure of Daeniker et al. (1972) and had similar GLC retention properties to the corn root unknown. The mass spectrum, though, of the α-chamigrene authentic sample measured on the same mass spectrometer, was clearly different from that of the unknown. The mass spectrum of an authentic sample of α-humulene was also clearly different.

The mass spectrum of the compound identified as bazzanene was consistent with that published (cf. Matsuo, 1971; Matsuo et al., 1973). Bazzanene has a very characteristic mass spectrum unlike that of any other sesquiterpene. The Kovats' GLC index for the unknown relative to that of β-caryophyllene as an internal marker was also consistent with that published for bazzanene by Anderson et al. (1977).

Growing and Isolation Conditions. No consistent difference was apparent in the volatiles found in the different varieties, the different growing locations, or in those found in relatively young plants (ca. 60 cm high) compared

to those of mature plants (ca. 2 m high). The use of different growing locations decreases the slight possibility that the compounds could be associated with the soil attached to the roots. The authors had also previously studied the volatiles of the soil in the general area where the corn was grown in Berkeley and had not detected any sesquiterpenes.

For the isolation the authors cut the roots to allow them to fit through the neck of the flask. This, of courses, causes damage to some cells and could possibly produce some volatiles (e.g. hexanal) not present in the intact plant. However, in the authors' previous experience with the roots of other plants (e.g., Kamm and Buttery, 1984) damage to root cells produces very little volatiles in contrast to damage to leaves or fruit where cell damage volatiles can be 100 times those present in the intact plant material.

Known Attractants. Ladd et al. (1983) had found that eugenol is an attractant for the adult northern corn root worm (*Diabrotica barberi* Smith and Laurence). Eugenol was actively searched for in the corn roots but could not be detected. It also had not been detected in other parts of the corn plant (cf. Buttery and Ling, 1984). It is interesting that one of the authors had recently identified methyleugenol in the roots of another major crop, red clover (Kamm and Buttery, 1984).

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LITERATURE CITED

Andersen, N. H.; Bissonett, P.; Liu, C. B.; Shunk, B.; Ohta, Y.; Tseng, C. W.; Moore, A.; Huneck, S. *Phytochemistry* **1977**, *16*, 1731.

Buttery, R. G.; Ling, L. C. *J. Agric. Food. Chem.* **1984**, *32*, 1104.

Daeniker, H. U.; Hochstetler, A. R.; Kaiser, K.; Kitchens, G. C. *J. Org. Chem.* **1972**, *37*, 1.

Heller, S. R.; Milne, G. W. A.; Eds. "EPI/NIH Mass Spectral Data Base"; U.S. Government Printing Office: Washington, D. C., 1978.

Kamm, J. A.; Buttery, R. G. *Environ. Entomol.* **1984**, *13*, 1427.

Ladd, T. L.; Stinner, B. R.; Krueger, H. R. *J. Econ. Entomol.* **1983**, *76*, 1049.

Matsuo, A. *Tetrahedron* **1971**, *27*, 2759.

Matsuo, A.; Nakayama, M.; Hayashi, S. *Bull. Chem. Soc. Jpn.* **1973**, *46*, 1010.

Smedman, L.; Zavarin, E.; Teranishi, R. *Phytochemistry* **1969**, *8*, 1457.

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Growth and Intrinsic Labeling of Peanuts with ^{65}Cu for Use in Human Bioavailability Studies

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Peanuts (*Arachis hypogaea* L.) were grown under greenhouse conditions. After pegging was extensive, each plant was stem-injected with 1.5 mg of ^{65}Cu (a stable isotope). After harvesting, the enrichment was 91 atom % of ^{65}Cu. These peanuts will be used in a copper bioavailability study with human subjects.

INTRODUCTION

Copper, an indispensable trace metal in human metabolism, is widely distributed in foodstuffs. The richest food sources of copper are shellfish, nuts, organ meats, and legumes (Pennington and Calloway, 1973). A daily coppper intake of 2–3 mg is recommended for adults (National Academy of Sciences, 1980), but dietary surveys show that actual intake is sometimes much lower, even below 1 mg/day (Klevay, 1975). Thus, the availability of copper for absorption from food is an important factor in the nutritional adequacy of the diet. Information on the bioavailability of copper from foods is very limited. Recently Lo et al. (1984) demonstrated that copper was equally available to rats from isolated soy protein and copper carbonate. Absorption of copper as $CuCl_2$ from purified diets (Turnlund, 1984; Turnlund et al., 1982; King et al., 1978) and in the fasting state (Johnson, 1984) has been studied in humans, but the availability of copper to humans from foodstuffs is virtually unknown. Copper has only two short-lived radioisotopes, ^{64}Cu, $t_{1/2}$ = 12 h, and ^{67}Cu, $t_{1/2}$ = 61.9 h. Thus only the stable isotope, ^{65}Cu, is of use for studies of copper absorption by humans. In addition, the ethical considerations which apply to radioisotope use in humans are not a concern when stable copper is used.

Peanuts (*Arachis hypogaea* L.) are relatively high in copper (7–8 ppm) and could constitute a good source of dietary copper. The phytate in legumes inhibits absorption of some trace minerals (O'Dell and Savage, 1960; Atwal et al., 1980). Spanish peanuts contain 1.88% phytic acid (Graf and Dintzis, 1982), a fairly high concentration. However, a study using extrinsically labeled peanuts fed to rats showed fairly high (41%) Cu absorption (Johnson et al., 1985), compared to 46% absorption of $CuSO_4$.

It is of special interest to determine whether copper intrinsic to a foodstuff is absorbed in the same manner as copper added extrinsically to a meal as a salt. It is well-known that extrinsic and intrinsic nonheme iron form a common pool in the gastrointestinal tract and are absorbed in approximately equal amounts (Consul and Lee, 1983). Extrinsic labeling for Zn, Cd, and Mg also appears to be a valid procedure (Evans and Johnson, 1977; Janghorbani et al., 1982; Welch and House, 1980; Schwartz et al., 1981). However, extrinsic labeling is not a valid technique for all elements (e.g., Se) (Siewicki and Balthrop, 1983). The validity of this procedure must be tested for each mineral. For these reasons, a study was initiated to determine the feasibility of intrinsically labeling peanuts with a stable isotope of copper, ^{65}Cu.

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