Occurrence and Assay of Estragole in the Leaves of Various Avocado Cultivars

Jimmie R. King* and Robert J. Knight

Estragole [1-methoxy-4-(2-propenyl)benzene] has been identified as a major constituent of the essential oil in the leaves of many cultivars of avocados. Methods for the assay of estragole were developed using either direct extraction or separation by steam distillation with subsequent quantification by gas–liquid chromatography (GLC) using a flame ionization detector. The leaves of 43 cultivars were collected and assayed for estragole because of its insecticidal properties and usefulness in chemotaxonomy. Estragole was found in the leaves of all cultivars of definite Mexican origin and in some cases exceeded 1% by weight of the fresh leaves. Most leaves from cultivars commonly assigned to the Mexican horticultural race, regardless of the actual place of origin, contained easily detectable amounts of estragole whereas estragole was not detectable in the leaves of cultivars of the West Indian or Guatemalan horticultural races. GLC data and partition coefficients are presented for estragole and two related naturally occurring compounds, safrole and anethole. Mass spectrometry and thin-layer chromatography were used to confirm the identity of estragole.

Estragole [1-methoxy-4-(2-propenyl)benzene; *p*-allylanisole] occurs in the essential oils of many plants used for flavoring and fragrances. The occurrence of estragole in the essential oil of avocado leaves was reported in a study comparing the leaf terpenes in 10 avocado cultivars and 2 closely related species by Bergh et al. (1973). The occurrence of estragole in the essential oil of the avocado, *Persea americana* Mill. var. drymifolia, has also been reported by Acosta de Iglesias et al. (1976). On the basis of what was earlier thought to be their respective centers of origen, three botanically distinguishable groups of *P. americana* were divided into three horticultural races, namely West Indian, Guatemalan, and Mexican races, and individual cultivars were assigned to a race by leaf scent, fruit skin thickness, season of ripening, and several other traits (Popenoe, 1920). The criteria used to determine the race were recently summarized by Bergh et al. (1973). A comprehensive monograph on the taxonomy of the genus *Persea* in the western hemisphere has been published by Kopp (1966). The origin, production, botany, and chemical constituents of avocados have recently been reviewed (Knight, 1980; Ahmed and Barmore, 1980). Most of the chemical assays reported involved lipids, waxes, nutrients, and flavor components. The principal criteria used to assign avocados to the Mexican race were anise-scented leaves and thin-skinned fruit. The anise scent of avocado leaves is shown here to be due to estragole and not the similarly scented anethole, which is the major component of the oil of anise and, as reported by Bergh et al. (1973), is a minor component of the leaf essential oil of some avocados. The insecticidal properties of 10 components of anise, including estragole, have recently been studied (Marcus and Lichtenstein, 1979). In the course of testing many compounds at this location as possible fumigants against the Caribbean fruit fly, *Anastrepha suspensa* (Loew), estragole was found to have moderate insecticidal properties (Benschoter et al., 1986).

The present work was initiated because of the insecticidal properties of estragole and for possible use in chemotaxonomy studies. The ready availability of many avocado cultivars at the Miami USDA plant introduction state greatly facilitated this research. The assigned racial lineages were developed from plant records at this station and agree with the assignments in a recent cold hardiness study (Malo et al., 1977). That study, and earlier work referenced there, indicates that avocado trees of pure and hybrid Mexican races are more cold tolerant.

Subtropical Horticultural Research Station, U.S. Department of Agriculture—Agricultural Research Service, Miami, Florida 33158.

MATERIALS AND METHODS

Gas Chromatography. Gas–liquid chromatography (GLC) data were obtained on a Hewlett-Packard Model 5880A instrument equipped with a level 4 terminal for instrument control and peak analysis, a flame ionization detector and a 180 cm × 4 mm (i.d.) glass column packed with 10% Carbowax 20M on 80–100-mesh GasChrom Q. For assays the following conditions were used: nitrogen carrier gas, 60 mL/min; injection port, 180 °C; oven temperature, 160 °C; detector temperature, 300 °C. To test the linearity of the detector, standards of estragole in hexane were prepared at concentrations of 2, 1, 0.5, 0.1, and 0.01 mg/mL and injections of 5 μL were made with a Hamilton syringe with a Chaney adapter. Linear regression analysis of the area vs. concentration gave a correlation coefficient of 0.99998, indicating excellent linearity. The peak areas of estragole in samples were compared to standards of similar, i.e. ±50%, concentration. Temperature programming was used in the determination of retention indexes with initial temperature 50 °C, 0-min hold time, 8 °C/min program rate, and final temperature 200 °C. In order to be consistent with other data from this laboratory, retention index calculations were made by cubic spline interpolation as described by Halang et al. (1978) except the algorithms were programmed in Basic for an IBM PC-XT computer. Linear interpolation gives the same results, to the nearest index unit, for these compounds.

Mass Spectra. A Finnigan MAT Model 700 ion trap detector in conjunction with a Hewlett-Packard Model 5710A gas chromatograph equipped with a 30-m capillary column (0.32-mm i.d., 1-μm DB5, from J&W Scientific, Inc.) was used to confirm the identity of estragole. Temperature programming was used as follows: 50 °C initial, 0 time hold, 8 °C/min program rate, 250 °C final temperature for 2 min. Helium carrier gas was used at 1 mL/min column flow with a 50:1 split ratio with a 5-μL injection.

Extraction Procedure. A 5-g leaf sample was weighed into a homogenizer flask (VirTis 6513-0205), 50 mL of a chloroform–methanol mixture (90:10) was added, and the

mixture was blended on a VirTis Model 25 homogenizer. The blended mixture was then filtered through a 25-mm bed of anhydrous sodium sulfate contained in a 50-mm glass funnel into a 70-mL culture tube. The filtrate was then assayed by GLC as described.

Steam Distillation Procedure. Mature leaves were collected and assayed as follows: A 5-g sample of leaf was weighed into a 500-mL Eberbach blending container, and 20 mL hexane and 100 mL of distilled water were added. The container was sealed with a Teflon-lined lid, and the contents were blended 1 min on the low speed of a Waring Blendor (No. 70105). The mixture was then poured through a 10-cm powder funnel into a 1000-mL round-bottom flask. An additional 150-mL portion of water was used to rinse the container, lid, and funnel into the flask. The flask was placed in a Glas-col heating mantle (No. TM108), and a 75 connecting tube (Kimble 44920), a 300-mm Liebig condenser (Kimble, 18130), a connecting tube (Kimble, 45005), and a round-bottom 100-mL receiving flask were connected in the order listed. The receiving flask was immersed in an ice–water bath to prevent the loss of hexane during the 20–30 min required for each distillation. A variable transformer was used to regulate the heating to obtain a rapid rate of distillation with no carryover of solid material. The distillation was continued until about 70 mL of distillate was collected: i.e., about 20 mL of hexane and 50 mL of water. The mixture was transferred to a 125-mL separatory funnel, and 5 g of sodium chloride was added. The mixture was thoroughly shaken and then allowed to separate. The water was removed, and a portion of the hexane was collected in a 12-mL culture tube containing about 1 g of anhydrous sodium sulfate and sealed with a Teflon-lined cap. The hexane layer was then assayed by GLC as described.

Reagents. Estragole (98%, A2,920-B), safrole (98%, S20-8), and anethole (11,787-0) were purchased from Aldrich Chemical Co. and used as received. Hexane, chloroform, and methanol were either nanograde or distilled in glass quality from Mallinckrodt, Inc., or Burdick & Jackson, Inc., respectively. No solvents exhibited interfering peaks. Distilled water was redistilled through a packed column; the first 10% was discarded, and the following 80% was collected and used. Because impurities can be concentrated during the steam distillation procedure, highly purified water is required.

RESULTS AND DISCUSSION

Initial identification of estragole in avocado leaves was made by thin-layer chromatography and gas–liquid chromatography. The closely related compounds anethole [1-methoxy-4-(1-propenyl)benzene] and safrole [5-(2-propenyl)-1,3-benzodioxole] were included in the initial studies. Under the isothermal GLC conditions described, a retention time of 4.10 min was obtained for both the standard and the unknown. The p values (fraction of solute partitioning into the least polar phase of a binary solvent system) for the three compounds were determined with use of equilibrated hexane and acetonitrile partitioning solvents by the method of Bowman and Berosa (1966). Using three replicates the values obtained, with standard deviations in parentheses, were 0.352 (0.001), 0.355 (0.001), and 0.271 (0.004) for estragole, anethole, and safrole, respectively. Mass spectral data obtained on the Finnigan ion trap detector confirmed the previous findings by giving an excellent match for spectra obtained from the estragole standard, the leaf extract compound, and the National Bureau of Standards Library Compilations spectra. The mass spectra of estragole and anethole [1-methoxy-4-(1-propenyl)benzene], which is different from estragole only in the location of the double bond in the side chain, were almost identical. However, the two compounds were readily separated on both the DB5 capillary column and the Carbowax 20M packed column. The retention times of the leaf compound were identical with those of estragole for all columns.

Table I. Mixed-Solvent Extraction of Avocado Leaf Estragole[a]

cultivar	no.[b]	race	amt
Akbal	M-00654	G	nd
Itzamna	M-00401	G	nd
Kanan	M-00649	G	nd
Kayab	M-00660	G	nd
Lamat	M-00646	G	nd
Nabal Seedling	M-20713	G	nd
Tertoh	M-00650	G	nd
Dade	M-00673	WI	nd
Donaldson	M-20024	WI	nd
Dora	M-20025	WI	nd
Fuchs	M-19851	WI	nd
Family	M-03895	WI	nd
Pollock	M-19846	WI	nd
Reulhe	M-19848	WI	nd
Rual Arango	M-20029	WI	nd
Titoperla	M-20031	WI	nd
Utuado	M-19768	WI	nd
Waldin	M-19850	WI	nd
Wester	M-00675	WI	nd
Wilson Popenoe	M-20032	WI	nd
Campanas	M-23014	M	lg
Chapultepec Park	M-18249	M	lg
Del Oro 2	M-18106	M	sm
Guayabamba	M-24544	M	sm
Morocco 43	M-19328	M	sm
Mexicola Sdlg. 1	M-17459	M	lg.
Romaine 1	M-19329	M	xsm
Young 1	M-20728	M	lg
Younghans	M-18571	M	lg
Capac	M-00641	M[c]	sm
Carchi	M-00635	M[c]	xsm
Chota	M-26355	M[c]	xsm
Egas	M-00640	M[c]	nd
Reina Victoria	M-24535	M[c]	sm
Antigua Market	M-11939	G × M	nd
Ettinger	M-14304	G × M	nd
Winter Mexican	M-18260	G × M	nd
Tamayo	M-00638	M × WI	nd
Gripina 5	M-19765	G × WI	nd
Simon	M-19853	G × WI	nd
Tonnage	M-19847	G × WI	nd
Irwing 120	M-20718	G × M	nd

[a] Key: nd = less than 0.005%; xsm = below 0.1%; sm = below 0.5;% lg = above 0.5%. [b] Accession number used by USDA—ARS at Miami. [c] From Ecuador; see text.

The leaves from 43 cultivars of avocados were collected and assayed by the mixed-solvent extraction procedure. The results are listed in Table I. This rapid method was used only as a screening procedure because recovery tests using leaves from Young 1 indicated a recovery of only 86% for estragole when compared to the steam distillation method. To determine the percent recovery by the steam distillation procedure duplicate samples of leaves from four cultivars were fortified with estragole and assayed. The results ranged slightly above 100% because some hexane is lost in the procedure whereas the calculated results are based on total recovery of the solvent. The recoveries, which varied only slightly with concentration, and standard deviations (in parentheses) based on eight replicates for samples fortified at levels of 0.01, 0.1, and 1.0% were 103.2 (1.9), 105.0 (1.4), and 106.6% (1.0), respectively. The leaves of all cultivars containing detectable amounts of estragole, plus a few others included for purposes of comparison, were assayed by the steam distillation method. A comparison

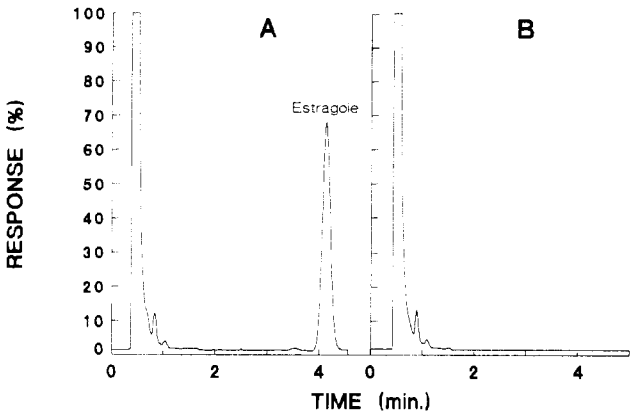

Figure 1. GLC chromatograms of steam distillation leaf extracts of Young 1 (A) and Simmonds (B) cultivars on a packed Carbowax 20M column using an FID detector (attenuation 1024) and GLC conditions as described in the text.

Table II. Steam Distillation Assay of Avocado Leaf Estragole

cultivar	race	no.[a]	amt,[b] % av ± SD
Atlisco 1	M	M-04043	0.032 ± 0.000
Chapultepec Park	M	M-18249	1.210 ± 0.024
Gauyabamba	M	M-24544	0.256 ± 0.002
Las Campanas	M	M-23014	1.078 ± 0.087
Morocco	M	M-19327	0.031 ± 0.001
Toltec	M	M-14768	nd
Young 1	M	M-20728	0.502 ± 0.030
Young 2	M	M-20729	0.505 ± 0.009
Capac	M[c]	M-00641	0.018 ± 0.001
Carchi	M[c]	M-00635	0.020 ± 0.002
Chota	M[c]	M-00639	0.021 ± 0.002
Gen. Francisco Robles	M[c]	M-24534	0.432 ± 0.002
Reina Victoria	M[c]	M-24535	0.286 ± 0.079
Simmonds	W.I.	M-00670	nd
Bacon	M × G	M-20709	0.142 ± 0.004
Winter Mexican	M × G	M-18260	nd
Fuerte	M × G	M-20711	0.078 ± 0.019
Mexicola Sdlg. 1	M × ?	M-17459	0.790 ± 0.004
Del Oro 1	M × ?	M-18105	0.033 ± 0.003
Del Oro 2	M × ?	M-18106	0.296 ± 0.012
Brooksville Sdlg.	M × ?	M-18686	0.666 ± 0.012
Brooksville Fl Sdlg.	M × ?	M-31764	nd
Brooksville Fl Sdlg.	M × ?	M-31765	0.051 ± 0.008
Brooksville Fl Sdlg.	M × ?	M-31766	nd
Brooksville Fl Sdlg.	M × ?	M-31767	0.010 ± 0.001

[a] Accession number used by USDA—ARS at Miami. [b] Average ± standard deviation of two samples. [c] From Ecuador; see text.

of the chromatograms for samples of Young 1 (Mexican race) and Simmonds (West Indian race) at attenuation 1024 is shown in Figure 1. The Simmonds sample gave no detectable peak even at 32× attenuation and therefore had less than 0.002% estragole based on the sensitivity of the integrator. The quantitative results, corrected for recovery, are shown in Table II. The probable racial lineage is included. From the data in Table I it is seen that, in general, avocados of Guatemalan or West Indian races have no detectable amounts of estragole whereas those of pure Mexican races all have easily detectable amounts. The present work lends support to the postulate that the Capac, Carchi, Chota, Gen. Francisco Robles, and Reine Victoria cultivars, collected at high elevations in Ecuador, belong to the Mexican race whereas the Egas cultivar does not. The absence of estragole in the leaves of the Toltec cultivar and the low level in the Morocco cultivar are unexpected in view of their proposed pure Mexican origen. The data in both Tables I and II indicate that crosses of Mexican cultivars with other cultivars can result in amounts of estragole in the leaves ranging from nondetectable to about 0.5% compared to over 1% in some pure Mexican cultivars. The results for the a Brooksville seedling and 4 F1 hybrids, shown at the bottom of Table II, illustrate this variability for crosses from a single plant and its seedlings. Further studies of the lineage of some of the cultivars and perhaps further chemical studies need to be done to assess the pertinence of these data to the taxonomy of avocados since chemotaxonomy is often a very useful adjunct to the more established methods and in some cases is the most reliable method. Also the levels of estragole in Mexican race avocado leaves are so high that they may well be of insecticidal importance.

ACKNOWLEDGMENT

We express our thanks to Gordon L. Millard for this technical assistance and to Irene Galvin for her help in procuring plant records.

Registry No. Estragole, 140-67-0; anethole, 104-46-1; safrole, 94-59-7.

LITERATURE CITED

Acosta de Inglesias, D. I.; De Viana, M. E. L.; Retamar, J. A. *Riv. Ital. Essenze, Profumi, Piante Off., Aromi, Saponi, Cosmet., Aerosol* **1976**, *58* (No. 3), 158.

Ahmed, E. M.; Barmore, C. R. *Tropical and Subtropical Fruits*; Nagy, S., Shaw, P. E., Eds.; AVI: Westport, CT, 1980; Chapter 2, pp 121–156.

Benschoter, C. A.; King, J. R.; McGovern, T. P. *Insecticide and Acaricide Tests* **1986**, *11*, 434.

Bergh, B. O.; Scora, R. W.; Storey, W. B. *Bot. Gaz. (Chicago)* **1973**, *134*(2), 130.

Bowman, M. C.; Berosa, M. *Anal. Chem.* **1966**, *38*, 1544.

Halang, W. A.; Langlais, R.; Kugler, E. *Anal. Chem.* **1978** , *50*, 1829.

Knight, R. J. In *Tropical and Subtropical Fruits*; Nagy, S., Shaw, P. E., Eds.; AVI: Westport, CT, 1980; pp 3–15.

Kopp, L. E. *Mem. N.Y. Bot. Gard.* **1966**, *14*, 1.

Malo, S. E.; Orth, P. G.; Brooks, N. P. *Proc. Fla. State Hortic. Soc.* **1977**, *90*, 247.

Marcus, C.; Lichtenstein, E. P. *J. Agric. Food Chem.* **1979**, *27*, 1217.

Popenoe, W. *Manual of Tropical and Subtropical Fruits*; MacMillan: New York, 1920; p 66.

Received for review September 22, 1986. Accepted May 4, 1987. Mention of a commercial or proprietary product does not constitute an endorsement by the USDA.