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Distinguishing Chinese Star Anise from Japanese Star Anise Using Thermal Desorption−Gas Chromatography−Mass Spectrometry

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The volatile compounds from the pericarps of *Illicium anisatum* L., *Illicium brevistylum* A.C.Sm., *Illicium griffithii* Hook.f. & Thomson, *Illicium henryi* Diels, *Illicium lanceolatum* A.C.Sm., *Illicium majus* Hook.f. & Thomson, *Illicium micranthum* Dunn, and *Illicium verum* Hook.f. were examined by thermal desorption−gas chromatography−mass spectrometry (TD-GC-MS). The volatiles desorbed from the pericarps of *I. verum* (Chinese star anise), the species traded for culinary purposes, were generally characterized by a high proportion of (*E*)-anethole (57.6−77.1%) and the presence of foeniculin; the latter was otherwise only detected in the pericarps of *I. lanceolatum*. In the pericarps of all other species analyzed, the percentage composition of (*E*)-anethole was comparatively lower (≤16.0%). The volatiles desorbed from the pericarps of the toxic *I. anisatum* (Japanese star anise) were characterized by the presence of asaricin, methoxyeugenol, and two other eugenol derivatives, none of which were detected in any of the other species examined. TD-GC-MS enables the direct analysis of the volatile components from the pericarps of *Illicium* and can assist with differentiating the fruits of *I. verum* from other species of *Illicium*, particularly the more toxic *I. anisatum*.

KEYWORDS: Star anise; *Illicium*; thermal desorption; gas chromatography−mass spectrometry; GC-MS; essential oil; anethole; fruit

INTRODUCTION

Star anise is defined as the dried composite fruit of *Illicium verum* Hook.f. (*1*). *I. verum* is also known as Chinese star anise, and it is widely used as a condiment for culinary purposes and as an infusion for its reputed sedative and carminative properties. In contrast, consumption of "false" star anise, *Illicium anisatum* L. (synonyms *Illicium japonicum* Sieb. and *Illicium religiosum* Sieb. & Zucc.; also known as Japanese star anise or shikimi fruit), has been associated with serious adverse effects including emesis and diarrhea, bradycardia, hallucinations, rhabdomyolysis, and convulsions (*2*−*4*). There is confusion over the common names for "star anise" (*5*) and, because of their similar morphology, misidentification of Chinese and Japanese star anise (*I. verum* and *I. anisatum*, respectively) can easily occur. Several reports have been documented in Europe and America that describe clinical toxicity, particularly neurological adverse effects, in both adults and infants that have consumed star anise often prepared as teas, and in some cases, the observed adverse effects could be attributed to contamination of *I. verum* with *I. anisatum* (*2*). Since the U.S. Food and Drug Administration (FDA) has received reports of seizures and other neurological effects associated with the consumption of adulterated Chinese star anise, the FDA issued a warning advising consumers not to drink teas prepared from star anise fruits (*2*). It is therefore essential that *I. verum* fruits are distinguished from the fruits of more toxic species of *Illicium* prior to their use in food products, and appropriate analytical methods to achieve this aim are required.

Sesquiterpene lactones have been isolated from a number of species of *Illicium* (Illiciaceae), and some of these compounds have been associated with neurotoxicity. Anisatin is a *seco*-prezizaane sesquiterpene isolated from the seeds and carpels of *I. anisatum* (*6*) and also from the fruits and leaves of *Illicium floridanum* J.Ellis (*7*) and the pericarps of *Illicium merrillianum* A.C.Sm. (*8*). Anisatin antagonizes the action of γ-aminobutyric acid (GABA) by acting as a noncompetitive antagonist of $GABA_A$ receptors (*9*, *10*). Neurotoxic sesquiterpene lactones, such as anisatin and neoanisatin, may explain the pharmacological basis of the adverse effects associated with the consumption of *I. anisatum* (*11*). Fruits of *Illicium majus* Hook.f. & Thomson are also reported to be toxic since neomajucin and 2-oxo-6-dehydroxyneoanisatin, sesquiterpene lactones isolated from the pericarps, have been associated with convulsant effects in vivo (*12*, *13*). Although some sesquiterpene lactones isolated from *I. verum* (veranisatins A, B, and C) are reported to be neurotoxic and to induce convulsions, they are considered to be less pharmacologically active than anisatin and occur at relatively low concentrations as compared to anisatin in *I. anisatum* (*3*, *14*, *15*).

The toxic sesquiterpene lactones can be detected using high-performance liquid chromatography−tandem mass spectrometry (*16*), and this method can be used to monitor whether potentially toxic star anise is entering the trade. It complements other methods to evaluate the quality and authenticity of star anise based on sensory, macroscopic and microscopic

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characteristics of the fruit, and thin-layer chromatography analysis and gas chromatography (GC)−flame ionization detection analysis of the essential oil (*1*, *3*, *16*−*18*). GC is regarded as a suitable method to assess the quality and authenticity of *I. verum* essential oil (*1*, *17*), and several studies have described the oil composition (*16*−*20*). The major component of *I. verum* oil is (*E*)-anethole, and the percentage content for (*E*)-anethole specified in the British Pharmacopoeia monograph (European Pharmacopoeia monograph 2108) for steam-distilled star anise oil is 86.0−93.0% (*1*). In contrast, the essential oils from few other species of *Illicium* have been studied in detail. The essential oils obtained from fruits of *I. anisatum* and *Illicium griffithii* Hook.f. & Thomson have been investigated, and the (*E*)-anethole content occurs at < 3.0% (*16*, *20*−*25*).

Limitations of the standard GC analysis of steam-distilled oil are that relatively large quantities of fruit material are required to obtain the oil and steam distillation is time-consuming. In some circumstances, the quantity of star anise fruit material available for analysis may be limited, or analytical data to determine authenticity may need to be obtained rapidly. Solvent extraction can be a faster technique to extract the essential oil components and requires less plant material, but comparative data on expected compositions are lacking.

The aim of this study was to address these issues by assessing the use of thermal desorption−gas chromatography−mass spectrometry (TD-GC-MS) as a simple and rapid means to authenticate and evaluate the quality of star anise fruits, without the need for steam distillation or sample preparation. An emphasis was placed on distinguishing Chinese star anise fruits (*I. verum*), which are commonly used in food, from those of the more toxic but morphologically similar Japanese star anise (*I. anisatum*). We also used TD-GC-MS to evaluate differences in essential oil composition of fruits from six other species of *Illicium* (*Illicium brevistylum* A.C.Sm., *I. griffithii*, *Illicium henryi* Diels, *Illicium lanceolatum* A.C.Sm., *I. majus*, and *Illicium micranthum* Dunn). The TD-GC-MS method was then applied to verify whether star anise fruits in a consignment seized by customs officers in the United Kingdom were from *I. verum*.

MATERIALS AND METHODS

Samples. Fruits from different species of *Illicium* were obtained from the Economic Botany Collections (EBC), Royal Botanic Gardens, Kew, United Kingdom, which includes collections of the Chinese Medicinal Plant Authentication Centre (CMPAC); the National Center for Natural Products Research (NCNPR), University of Mississippi, United States; the Tsukuba Botanical Garden (TBG), National Science Museum, Tsukuba, Japan; and the American Herbal Pharmacopoeia (AHP) (**Table 1**). A trade essential oil sample labeled as *I. verum* (sample BI 12735) was also obtained for analysis. Fruits from two batches of star anise seized by customs officers in the United Kingdom were also examined; six fruits were selected from each batch (samples BI 10295 A−F and BI 12249 G−L).

GC-MS and TD-GC-MS Parameters. The TD-GC-MS system consisted of an ATD 400 thermal desorption unit, an AutoSystem XL GC, and a TurboMass quadrupole MS (Perkin-Elmer, Waltham, MA). Approximately 2 mg of pericarp was held between glass wool in a homemade glass tube insert that fit into the manufacturer's stainless steel desorption tube. The sample was desorbed at 150 °C for 10 min in a flow of 60 mL/min helium that passed to a Tenax TA trap (80−100 mesh) held at 4 °C with no inlet split. Following desorption, the Tenax trap was heated ballistically to 300 °C under a helium pressure of 15 psi and with an outlet split flow of 18.75 mL/min. The volatile components passed through a deactivated glass capillary transfer line at 200 °C onto a 30 m × 0.25 mm i.d. × 0.25 μm DB-5MS capillary GC column (Agilent J&W, Santa Clara, CA), and chromatography proceeded using an oven temperature program of 60−300 at 6 °C/min under the pressure from the ATD. The MS was fitted with an EI source operated at 70 eV with a source temperature of 180 °C, and mass spectra were recorded in the range m/z 38−300. The operating software was Turbomass version 4.1.1. Retention indices (RI) were determined in relation to a series of *n*-alkanes (C8−C20, Supelco, United Kingdom), and peak integration was performed to RI 1900 (i.e., prior to the elution of palmitic acid). Compounds were identified by comparing RI and/or mass spectra with published data (*26*, *27*). To assess the variation in essential oil composition in different sections of pericarp, 2 mg fragments from different sections of one pericarp from *I. verum* ($n = 6$) and one pericarp from *I. anisatum* ($n = 6$) were analyzed by TD-GC-MS as described above.

An initial experiment investigated the effect of different thermal desorption parameters by desorbing 2 mg pericarp fragments of a fruit of *I. verum* at 80, 100, 150, or 200 °C for 3 or 10 min. The TD-GC-MS analyses were compared to that obtained from trade star anise oil by split liquid injection GC-MS using the same column and an oven temperature program of 40−300 at 3 °C/min; the oil was diluted 1/100 in diethyl ether (Fisher Scientific), and 1 μL (split 1:10) was injected at 220 °C by an autosampler.

RESULTS AND DISCUSSION

Selection of Thermal Desorption Conditions. Tests using TD-GC-MS were performed on fragments of pericarp from a fruit of *I. verum* to determine suitable desorption temperatures and times. The relative amounts of (*E*)-anethole, estragole, and foeniculin following desorption of 2 mg of *I. verum* pericarp using eight desorption conditions are listed in **Table 2**. These compounds are three of the main constituents reported to occur in *I. verum* oil (*1*, *17*).

At a desorption temperature of 200 °C, levels of higher boiling point compounds such as fatty acids were markedly increased in the desorbed volatiles, which necessitated the baking of the GC column between analyses to clean it. Polar organic acids were also desorbed at 200 °C, and their poor chromatography on the nonpolar column interfered with peak integration. Desorption conditions of 150 °C for 10 min were chosen for subsequent analyses as these provided a compromise between mimicking steam distillation (i.e., the desorption of compounds that are detected in *I. verum* oils obtained by steam distillation) and the detection of compounds with high RI values that subsequently proved important in the discrimination of fruits from *I. verum* and *I. anisatum*. The percentage composition of volatiles desorbed from an *I. verum* pericarp under the selected TD-GC-MS conditions is compared to that of a steam-distilled oil analyzed by liquid injection GC-MS in **Table 3**.

TD-GC-MS of Fruit from *I. verum* as Compared to *I. anisatum* and Other *Illicium* Species. (*E*)-Anethole (36.5−81.3%) was the major component desorbed from the fruit pericarps of *I. verum* obtained from the CMPAC (EBC) that had been acquired between 2001 and 2002 (**Table 4**). In contrast, for recently collected fruit of *I. anisatum*, the level of (*E*)-anethole desorbed from pericarp fragments was ≤1.8%. This is in accordance with published analyses of the essential oil of *I. anisatum* in which (*E*)-anethole is reported at < 2.0% (*16*, *22*), much lower than in *I. verum* oil (*1*, *16*). However, when fruit from the historic holdings of the EBC were analyzed by TD-GC-MS, the range of (*E*)-anethole content was found to be similar for both species (**Table 4**), suggesting that differentiating *I. verum* from *I. anisatum* on the basis of (*E*)-anethole content alone could become more unreliable when analyzing fruit stored for long periods. In recently collected fruits of other *Illicium* species, relative levels of (*E*)-anethole desorbed from pericarp fragments were also low: ≤ 6.7% in *I. lanceolatum*, ≤ 1.1% in *Illicium brevystylum*, < 0.1% in *I. henryi*, and ≤3.0% in both *I. majus* and *I. micranthum*. *I. griffithii* fruits were only available from the historic collections (EBC), dating from either the 19th or the early 20th century, and (*E*)-anethole was only detected at 0.3 and 0.4% in the two fruits available for analysis. In published studies on the fruit essential oil

Table 1. Collection Details for Fruits of Species of *Illicium*

Illicium species	sample no./collection reference	acquisition date	collection details
I. verum[a]	BI 12218 EBC 75871	donated 1998 via the Leather Conservation Centre (collection amassed end of C19, beginning of C20)	origin unknown
I. verum[a]	BI 12223 EBC 40948	estimated 1875−1876	India
I. verum[a]	BI 12224 EBC 40944	unknown	China
I. verum[a]	BI 12225 EBC 40942	donated 1891	Hong Kong
I. verum[a]	BI 12226 EBC 42063	donated 1983, collected C19 and early C20	China
I. verum[b]	BI 10246 EBC 80978	12/02/02	market sample (origin unknown)
I. verum[b]	BI 10247 EBC 80964	bought 21/01/02	market sample (origin unknown) bought in China town, London
I. verum[b]	BI 10248 EBC 80977	5/02/02	market sample (origin unknown) provided by London TCM trader
I. verum[b]	BI 10800, BI 13383 (n = 2) EBC 81141[f]	harvested 14/09/01	Guangxi province, China; steamed and then sun-dried
I. verum[b]	BI 10801 EBC 81135	bought 12/09/01	TCM clinic in Guangxi province, China
I. verum[b]	BI 10803 EBC 81136	bought 12/09/01	market sample from Guangxi province, China
I. verum[b]	BI 11547 EBC 81468	bought 20/06/02	market sample from a pharmacy, Paris, France (origin unknown)
I. verum[b]	BI 11548 EBC 81411	7/01/02	China (source unknown)
I. verum[b]	BI 11549 EBC 81397	bought 10/01/01	China (source unknown)
I. verum[b]	BI 13395 EBC 80967	23/01/02	Kent County Council (source unknown)
I. anisatum[a]	BI 12217 EBC 42018	collected 1955, donated 1983	Malaysia
I. anisatum[a]	BI 12219, BI 10296 (n = 2) EBC 40946	donated 1882	Japan
I. anisatum[a]	BI 12221 EBC 42016	sample EBC 42016 or sample EBC 42050 may have been collected in 1881, donated 1983 RPSGB collection: drugs collected C19 and early C20	Japan
I. anisatum[a]	BI 12228 EBC 42050	sample EBC 42016 or sample EBC 42050 may have been collected in 1881, donated 1983 RPSGB collection: drugs collected C19 and early C20	Japan
I. anisatum[c]	BI 14685 Coll. No. 1059 and 1073	September 2005	Japan, voucher specimens deposited at the National Center for Natural Products Research, University of Mississippi, as described by ref (*28*)
I. anisatum[d]	BI 14814	October 2005	Japan, unprocessed fruit freeze-dried at RBG Kew (Nov 2005)
I. anisatum[d]	BI 14814	October 2005	Japan, unprocessed fruit oven-dried at 60 °C at RBG Kew (Nov 2005)
I. anisatum[d]	BI 14824	November 2005	Japan
I. brevistylum[e]	BI 14347 reference 645	July 2005	China, identified by Wang Zhong Dong, Henan Province
I. griffithii[a]	BI 10236 EBC 42049	donated 1983, collected C19 and early C20	India
I. griffithii[a]	BI 12222 EBC 40950	donated 1859	Malaysia
I. henryi[e]	BI 14348 reference 643	July 2005	China, identified by Wang Zhong Dong, Henan Province
I. lanceolatum[b]	BI 10802, BI 13384, BI 13385 (n = 3) EBC 81114[f]	harvested 26/10/01	fallen fruit from tree cultivated in the Botanic Garden of Hangzhou Institute of Medicine, Zhejiang Province, China; unprocessed and air-dried fruit
I. lanceolatum[b]	BI 11545, BI 13386 (n = 2) EBC 81210[f]	harvested 26/10/01	from same tree as described for voucher specimen EBC 81114, cultivated in the Botanic Garden of Hangzhou Institute of Medicine, Zhejiang Province, China; fruit harvested directly from tree, then steamed, and then oven-dried at 40 °C
I. lanceolatum[b]	BI 11546 EBC 81413	received at RBG Kew 1/07/02	Institute of Medicinal Plant Development (IMPLAD), Beijing, China
I. majus[a]	BI 12227 EBC 42048	donated 1983, collected C19 and early C20	origin unknown
I. majus[e]	BI 14349 reference 642	July 2005	China, identified by Wang Zhong Dong, Henan Province
I. micranthum[a]	BI 12220 EBC 40943	donated 1886	China
I. micranthum[e]	BI 14350 reference 644	July 2005	China, identified by Wang Zhong Dong, Henan Province

[a] Obtained from the collections at the Centre for Economic Botany (CEB), Royal Botanic Gardens (RBG) Kew. [b] Obtained from the collections at the Chinese Medicinal Plants Authentication Centre (CMPAC), Royal Botanic Gardens (RBG) Kew. [c] Obtained from the National Center for Natural Products Research (NCNPR), University of Mississippi, United States. [d] Obtained from the Tsukuba Botanical Garden (TBG), National Science Museum, Japan. [e] Obtained from the American Herbal Pharmacopoeia (AHP). [f] Harvested as authentic reference specimens and accompanied by herbarium vouchers (RBG Kew and IMPLAD); C, century; RPSGB, Royal Pharmaceutical Society of Great Britain; TCM, traditional Chinese medicine.

of *I. griffithii*, (*E*)-anethole is reported at $<3\%$, or it was not detected (*23*−*25*). Thus, as described previously (*1*, *16*, *17*), a high relative level of (*E*)-anethole provides an indication of *I. verum* authenticity and quality.

The detection of foeniculin could be useful in differentiating between fruits of *I. verum* and *I. anisatum*, since this component was detected in all recent (nontrade) and historic specimens of *I. verum* (0.6−21.1%), but it was not detected in any specimens of *I. anisatum*. Among the other species studied, foeniculin was only detected in the volatiles desorbed from two of the six fruits of *I. lanceolatum* analyzed (**Table 4**). Thus, the presence of foeniculin in individual fruits of star anise may indicate that a batch of *I. verum* fruit has not been adulterated with the fruit of *I. anisatum*, but it should be considered that foeniculin could also be an indicator of *I. lanceolatum* fruit. Estragole was detected among the desorbed volatiles of all fruits of *I. verum*, and in some,

Table 2. Relative Amounts of Estragole, (*E*)-Anethole, and Foeniculin Recorded from Pericarp Fragments of a Fruit of *I. verum* (BI 10295), Following TD-GC-MS Analysis Using Different Desorption Temperatures and Times (Based on All Peaks Integrated up to RI 1900), with Chromatography Performed on a 30 m × 0.25 mm i.d. × 0.25 μm DB-5MS Column

		relative amount (%)		
temp (°C)	time (min)	estragole	(*E*)-anethole	foeniculin
80	3	0.5	90.8	0.0
100	3	0.4	87.5	0.3
150	3	0.2	88.7	2.6
200	3	0.4	72.5	3.6
80	10	0.4	93.9	0.7
100	10	0.2	94.4	1.0
150	10	0.2	79.7	4.8
200	10	0.6	54.6	2.8

Table 3. Percentage Composition of Compounds Detected in an Essential Oil Labeled as *I. verum* (BI 12735) and the Thermally Desorbed Pericarp from *I. verum* (BI 10800), Obtained from the GC-MS Total Ion Chromatograms (Based on All Peaks Integrated up to RI 1900) with Chromatography Performed on a 30 m × 0.25 mm i.d. × 0.25 μm DB-5MS Column[a]

	KI		% composition	
compound	experimental	published	essential oil	desorbed pericarp
α-thujene	921	930	tr	ND
α-pinene	928	939	0.5	0.1
β-pinene	969	979	0.1	0.2
myrcene	982	991	tr	0.1
α-phellandrene	998	1003	0.1	0.2
cymene	1017	1025 (*p*-), 1026 (*o*-)	0.2	0.1
limonene	1022	1029	0.4	0.4
1,8-cineole	1025	1031	0.2	tr
γ-terpinene	1052	1060	tr	tr
linalool	1098	1097	1.4	0.7
terpinen-4-ol	1182	1177	0.2	tr
estragole	1202	1196	4.0	0.8
p-anisaldehyde	1262	1250	3.3	4.5
(*E*)-anethole	1302	1285	80.8	81.3
α-copaene	1379	1377	tr	0.2
cis-α-bergamotene	1416	1413	tr	0.2
(*E*)-caryophyllene	1421	1419	0.3	0.5
trans-α-bergamotene	1435	1435	0.3	0.4
aromadendrene	1438	1441	0.1	ND
(*E*)-cinnamyl acetate	1446	1446	tr	ND
(*E*)-methyl isoeugenol	1493	1492	tr	tr
(*E,E*)-α-farnesene	1502	1506	tr	tr
β-bisabolene	1504	1506	0.1	0.1
γ-cadinene	1508	1514	tr	tr
elemol	1541	1550	tr	tr
(*E*)-nerolidol	1553	1563	0.1	0.2
p-methoxy-cinnamaldehyde	1556	1564	tr	0.1
spathulenol	1564	1578	tr	0.7
caryophyllene oxide	1568	1583	tr	ND
α-cadinol	1633	1654	0.1	0.1
foeniculin	1652	1678	1.5	3.5

[a] tr, trace (< 0.1%); ND, not detected. Compounds were identified by comparing retention indices (calculated against an *n*-alkane series) and by comparing mass spectra with published data (*26*, *27*).

it was an abundant component (≤49.1%), but low levels were also detected in some fruits of *I. anisatum* (≤0.5%) and in the one available historic fruit of *I. micranthum* (< 0.1%).

Among other components, those that have been considered as markers for *I. anisatum* include eugenol, methyleugenol, methoxyeugenol, safrole, and myristicin (*2*, *20*−*22*, *28*). Using TD-GC-MS, it was found that methyleugenol and myristicin were not reliable features of the desorbed volatiles of *I. anisatum* pericarps, as they were detected in only seven and four of 26 fruits analyzed, respectively. Among the other *Illicium* species studied, trace levels of methyleugenol were also detected in all of the *I. brevistylum* fruits, and both methyleugenol and myristicin were detected in five of the six *I. lanceolatum* fruits (< 0.1 and ≤2.1%, respectively).

Although safrole and eugenol were detected in 25 and 24 of the 26 pericarps of *I. anisatum*, respectively, they are not useful markers for *I. anisatum*, as TD-GC-MS analysis revealed their presence in some other *Illicium* species. Safrole was detected among examples of all of the other species studied except *I. verum* (not detected in any specimens) and the historical EBC specimens of *I. griffithii*. This supports the more recent findings of Lederer et al. (*16*) who, from essential oil analyses, found that safrole (and myristicin) was not restricted to *I. anisatum* but occurred also in the oils of *I. lanceolatum*, *I. henryi*, *I. micranthum*, and *I. simonsii* Maxim. Although safrole was not detected in the historical EBC specimens of *I. griffithii*, it has been reported as a major component in the steam-distilled oil from this species (*24*, *25*), occurring at over 50% in one analysis (*25*). In another study of the fruit essential oil of *I. griffithii*, only a trace amount of safrole (< 0.1%) was found, and the major components were α-pinene, linalool, limonene, and 1,8-cineole (*23*). The differences in the composition of the essential oil from *I. griffithii* fruit could be due to different phenotypes of *I. griffithii*, which have been reported to vary in their essential oil composition (*25*).

The absence of safrole and myristicin in the essential oil of Chinese star anise fruit has been suggested to indicate the absence of contamination of *I. verum* fruits with the fruits of other species of *Illicium* (*16*). However, using TD-GC-MS, the erratic detection of safrole among individual fruits from different *Illicium* species means that absence data may not reliably indicate the absence of adulterants among a batch of Chinese star anise. Lederer et al. also failed to detect safrole in some essential oil samples obtained from *I. anisatum* or *I. lanceolatum* fruits (*16*). Conversely, though, the detection of safrole in a fruit by TD-GC-MS would indicate that the sample was not *I. verum*, even though the compound is not a reliable marker for *I. anisatum* or for distinguishing *I. anisatum* from some other species of *Illicium*.

Eugenol should be dismissed as a possible marker for *I. anisatum* as it was also detected by TD-GC-MS in seven of 31 fruits analyzed of *I. verum*. Eugenol was also detected in all specimens of *I. lanceolatum* analyzed, all of the historical (EBC) samples of *I. majus*, and in one sample of *I. brevistylum*. Methoxyeugenol, however, as well as being consistently detected in all specimens of *I. anisatum*, was not detected among any of the fruits from the other *Illicium* species studied, and specifically, it was not detected in any examples of *I. verum*. Thus, methoxyeugenol remains a candidate marker component for *I. anisatum* and could be useful to distinguish fruits of *I. anisatum* from *I. verum* and some other species of *Illicium*.

The results of TD-GC-MS analyses indicated that three other compounds, in addition to methoxyeugenol, could be useful to distinguish fruits of *I. verum* from those of *I. anisatum*. These were asaricin and two compounds (**14** and **15a**) assigned as eugenol derivatives. These three compounds were detected in all recently collected fruits of *I. anisatum* (asaricin, ≤6.5%; **14**, ≤49.5%; and **15a**, ≤17.1%) but were not detected in the pericarps of any of the other *Illicium* species studied, including *I. verum*. The EI spectra of compounds **14** and **15a** showed fragment ions in common with eugenol and methoxyeugenol but with low abundance, likely molecular ions at *m*/*z* 232 and 262, respectively, in accordance

Table 4. Percentage Composition of Some Compounds Detected in the Pericarps from the Fruit of Species of *Illicium*, Obtained from TD-GC-MS Total Ion Chromatograms (Based on All Peaks Integrated up to RI 1900) with Chromatography Performed on a 30 m × 0.25 mm i.d. × 0.25 μm DB-5MS Column[a]

species of *Illicium*	source	*n*	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15a/15b***
I. anisatum	EBC	5	tr−0.4	3.9−9.6	ND−0.5	1.5−16.0	ND−1.0	ND−0.4	ND−0.2	2.0−4.7	tr−16.9	ND	ND	tr−1.2	ND	ND−11.8	ND−9.4
I. anisatum	NCNPR, TBG	12	tr−0.6	4.5−20.3	ND−tr	ND−1.8	4.9−28.5	0.2−0.6	ND−tr	3.5−15.6	tr−5.9	ND−1.9	ND	0.3−2.7	ND	1.3−49.5	1.1−16.5
I. anisatum (freeze-dried)	TBG	6	0.3−0.9	8.0−16.9	ND−tr	tr−0.5	14.4−27.4	tr−0.2	ND−tr	3.0−7.6	1.0−6.3	ND	ND	1.5−4.0	ND	1.4−3.3	3.6−17.1
I. anisatum (oven-dried at 60 °C)	TBG	3	0.5−1.2	13.6−18.4	ND	tr	15.6−23.9	0.2	ND−tr	4.9−7.3	0.4−6.5	ND	ND	0.6−3.5	ND	1.4−2.5	4.1−12.4
I. brevistylum	AHP	3	1.2−11.1	3.0−6.6	ND	tr−1.1	ND−tr	ND−tr	tr	tr−5.0	ND	ND	ND	ND	ND	ND	ND
I. griffithii	EBC	2	ND−tr	tr−0.1	ND	0.3−0.4	ND	ND	ND	14.9−17.3	ND	ND	tr	ND	ND	ND	43.8−57.2*
I. henryi	AHP	3	12.4−32.8	1.2−2.1	ND	tr	0.4−2.7	ND	ND	1.5−8.5	ND	ND	ND	ND	ND	ND	ND
I. lanceolatum	CMPAC	6	0.1−0.6	4.1−36.6	ND	tr−6.7	ND−0.2	tr	ND−tr	ND−0.8	ND	ND−2.1	tr−10.3	ND	ND−1.2	ND	ND
I. majus	EBC	1	0.1	ND	ND	1.3	ND	tr	ND	10.1	ND	ND	ND	ND	ND	ND	ND
I. majus	AHP	3	3.5−10.2	0.7−1.0	ND	tr−3.0	tr−1.7	ND	ND	0.5−6.9	ND	ND	ND	ND	ND	ND	ND
I. micranthum	EBC	1	1.1	0.3	tr	2.6	ND	ND	ND	0.9	ND	ND	ND	ND	ND	ND	ND
I. micranthum	AHP	3	2.9−17.9	0.5−1.6	ND	tr−2.2	1.0−1.5	ND	ND	2.9−8.6	ND	ND	ND	ND	ND	ND	ND
I. verum	EBC	5	0.2−0.6	0.1−1.4	0.4−49.1	3.7−16.7	ND	ND−tr	ND	ND−4.3	ND	ND	ND	ND	0.6−13.2	ND	ND
I. verum	CMPAC	14	tr−1.6	ND−0.2	0.1−1.1	36.5−81.3	ND	ND−tr	ND	ND−3.0	ND	ND	ND	ND	1.1−21.1	ND	ND
I. verum	traded	10	0.1−1.2	tr−0.6	tr−7.5	57.6−77.1	ND	ND	ND	0.1−1.1	ND	ND	ND	ND	0.8−22.4	ND	ND
I. verum (BI 10295 B)	traded	1	0.1	tr	19.3	29.7	ND	ND	ND	0.2	ND	ND	ND	ND	17.8	ND	ND
I. verum (BI 12249 L)	traded	1	ND	ND	0.2	5.1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

[a] Key: **1**, limonene (RI 1012); **2**, 1,8-cineole (RI 1017); **3**, estragole (RI 1190); **4**, (*E*)-anethole (RI 1290); **5**, safrole (RI 1295); **6**, eugenol (RI 1356); **7**, methyleugenol (RI 1403); **8**, (*E*)-caryophyllene (RI 1438); **9**, asaricin (RI 1517); **10**, myristicin (RI 1541); **11**, elemicin (RI 1568); **12**, methoxyeugenol (RI 1592); **13**, foeniculin (RI 1694); **14**, eugenol derivative (RI 1745); **15a**, methoxyeugenol derivative (RI 1847); and **15b***, methoxyeugenol derivative (RI 1821). tr, trace (< 0.1%); ND, not detected; EBC, Economic Botany Collections (Royal Botanic Gardens, Kew); NCNPR, National Center for Natural Products Research (University of Mississippi, United States); TBG, Tsukuba Botanical Garden (National Science Museum, Japan); AHP, American Herbal Pharmacopoeia; CMPAC, Chinese Medicinal Plants Authentication Centre (Royal Botanic Gardens, Kew).

Figure 1. GC-MS total ion chromatograms of thermally desorbed components of fruit pericarps from (**A**) *I. verum* (BI 10800) and (**B**) *I. anisatum* (BI 14685), using a 30 m × 0.25 mm i.d. × 0.25 μm DB-5MS column. Compounds: **1**, limonene; **2**, 1,8-cineole; **3**, estragole; **4**, (*E*)-anethole; **5**, safrole; **6**, eugenol; **8**, (*E*)-caryophyllene; **9**, asaricin; **12**, methoxyeugenol; **13**, foeniculin; **14**, eugenol derivative; and **15a**, methoxyeugenol derivative.

with prenylated derivatives of eugenol and methoxyeugenol. Compounds **14** and **15a** were higher boiling point compounds that were prominent among the volatiles desorbed from *I. anisatum* pericarps at 150 °C but have not been reported in steam distillates (*21*) or dichloromethane extracts (*28*) of *I. anisatum* fruit. *O*-Prenyl eugenol (1-allyl-3-methoxy-4-(3-methylbut-2-enyloxy)benzene [$M^+ = m/z$ 232]) has been isolated from the leaf oil of *I. anisatum* (*29*) and from the leaves of *I. tashiroi* Maxim. (*30*), and *O*-prenyl methoxyeugenol (1-allyl-3,5-dimethoxy-4-(3-methylbut-2-enyloxy)benzene [$M^+ = m/z$ 262]) has been isolated from the leaves and wood of *I. anisatum* (*29*, *30*). The detection of asaricin, **14**, and **15a** in TD-GC-MS analysis of fruits considered to be Chinese star anise would indicate that *I. verum* is adulterated with *I. anisatum*. Fruits from the historic collections of *I. griffithii*, however, contained high percentage compositions of a eugenol derivative (**15b**) (43.8 and 57.2%) that had a similar mass spectrum to **15a** but different RI, which might be a useful indicator of this species.

Thus, in authenticating the identity of fruits of *I. verum*, particularly to distinguish them from those of *I. anisatum*, important characteristics of the profile of volatiles observed following TD-GC-MS analysis are a high relative level of (*E*)-anethole, the presence of foeniculin, and the absence of safrole, asaricin, methoxyeugenol, **14**, and **15a**. Total ion chromatograms of the thermally desorbed components of fruit pericarps from *I. verum* (BI 10800) and *I. anisatum* (BI 14685) are shown in **Figure 1**. To investigate if any variation in essential oil composition occurs between different sections of the same pericarp, six different pericarp fragments from both *I. verum* and *I. anisatum* were assessed using the selected TD-GC-MS conditions. The data [expressed as the means ± standard deviations (SDs) for six compounds, three detected in each species] show the SD values ranged from 0.18 to 4.14 (**Table 5**), indicating some variation in essential oil composition in different fragments of the same pericarp from both *I. verum* and *I. anisatum*. This variation did not influence the ability to differentiate between fruits from *I. verum* and *I. anisatum*.

It is important to consider that other species of *Illicium* may have adverse effects when ingested. For example, *I. lanceolatum* fruit is reported to be toxic and to induce dizziness, nausea, vomiting, and convulsions (*3*, *31*). *Illicium arborescens* Hayata & Hayata, *I. brevystylum*, *I. henryi*, *Illicium macranthum* A.C.Sm., *I. majus*, *Illicium minwanense* B.N.Chang & S.D.Zhang, *I. simonsii*, and *I. ternstroemioides* A.C.Sm. are also reported to

Table 5. Variation in Desorbed Volatile Components from Different Fragments of One Pericarp from *I. verum* (BI 10800) and One Pericarp from *I. anisatum* (BI 14685), Expressed As Mean Percentage Composition ($n = 6$), with Chromatography Performed on a 30 m × 0.25 mm i.d. × 0.25 μm DB-5MS Column (Based on All Peaks Integrated up to RI 1900)

I. verum						*I. anisatum*					
estragole (%)		(*E*)-anethole (%)		foeniculin (%)		asaricin (%)		eugenol derivative (**14**) (%)		methoxyeugenol derivative (**15a**) (%)	
mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
0.30	0.21	53.25	4.14	6.18	1.59	0.57	0.18	5.42	0.92	2.55	0.84

be toxic (*3*, *12*, *13*). Thus, methods that assist with the detection of the less well-documented species of *Illicium* are also required.

Elemicin was a characteristic component of the volatiles desorbed from pericarps of *I. lanceolatum*, as compared to the other species studied by TD-GC-MS. It was detected in all six specimens of *I. lanceolatum* analyzed (≤10.3%) and was not detected in samples of any of the other species studied, except for trace amounts (< 0.1%) in the historic (EBC) *I. griffithii* fruits. Elemicin has also been reported to occur in the steam-distilled oil from *I. griffithii* fruits (*24*). Further investigation on the consistency of elemicin in the desorbed volatiles of *I. lanceolatum* fruit would be useful to distinguish this reportedly toxic species (*3*, *31*) from *I. verum*, given that foeniculin, seemingly a characteristic volatile component of *I. verum*, was also detected in two of the six pericarps of *I. lanceolatum* analyzed.

(*E*)-Caryophyllene was detected among the volatiles of all species studied, but relative levels were much higher in the two historic (EBC) fruits of *I. griffithii* (14.9 and 17.3%) and in one of the recently collected *I. anisatum* fruits (15.6%). Lower percentage compositions of this sesquiterpenoid were detected in the other 25 specimens of *I. anisatum* studied (≤8.3%) and in the specimens of *I. verum* (≤4.3%). Finally, limonene was detected at relatively high levels among volatiles desorbed from some of the pericarps of recently collected fruits from *I. brevistylum*, *I. henryi*, *I. majus*, and *I. micranthum*, whereas this component was only detected at ≤1.6% in the desorbed volatiles from *I. verum*.

TD-GC-MS of Traded Star Anise Samples. Some quantitative differences were observed in the composition of the essential oils thermally desorbed from the traded batches of star anise fruits when subjected to TD-GC-MS analysis (**Table 4**). In 10 of the 12 fruits (BI 10295 A, C−F and BI 12249 G−K), (*E*)-anethole was the major desorbed component from the pericarps, accounting for 57.6−77.1% of the monitored volatiles. This is within the range observed for the recently collected fruits of *I. verum* obtained from CMPAC (36.5−81.3% (*E*)-anethole) and exceeds the levels recorded for any other species analyzed in this study. The levels of (*E*)-anethole desorbed from the pericarps of the remaining two trade fruits were lower, 29.7% for fruit BI 10295 B and 5.1% for fruit BI 12249 L. Estragole was detected in all of the fruits traded as *I. verum*; levels were ≤7.5% except for the pericarp of fruit BI 10295 B in which 19.3% estragole was detected, considerably higher than recorded for the fruits of *I. verum* obtained from CMPAC (0.1−1.1%). Foeniculin was also detected in the pericarps of all of the trade fruits, except for fruit BI 12249 L. Thus, the TD-GC-MS chromatographic profile of 10 trade fruits with high (*E*)-anethole contents were typical of *I. verum*. The profiles of the two trade fruits with relatively low (*E*)-anethole levels still showed chemical characteristics more associated with *I. verum* than any of the other species of *Illicium* analyzed in the present study, indicating these two fruits are of low quality. Importantly, all of the traded fruits sampled lacked detectable levels of asaricin, methoxyeugenol, and compounds **14** and **15a**, which were associated with the fruits of *I. anisatum*.

In conclusion, TD-GC-MS provides a rapid, simple, and solvent-free method for the analysis of the volatile components from the fruit of species of *Illicium*. The need for extraction or distillation processes is eliminated, and only a small quantity of plant material is required (approximately 2 mg used in the present study). It is apparent that TD-GC-MS can assist with the differentiation between fruit obtained from *I. verum* and the more toxic *I. anisatum* and has potential applications for the authentication and differentiation among fruit from different species of *Illicium*. Therefore, by individually analyzing fruits at random by TD-GC-MS, the general quality as well as authenticity of a batch of fruits can be assessed relatively nondestructively. The technique would also be useful in helping to identify the species involved in circumstances, such as in a poisoning case, where the number of fruits available for analysis is limited and steam distillation would be difficult.

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LITERATURE CITED

(1) *British Pharmacopoeia*; The Stationery Office: London, United Kingdom, 2007; Vol. I.
(2) Ize-Ludlow, D.; Ragone, S.; Bruck, I. S.; Bernstein, J. N.; Duchowny, M.; Pena, B. M. G. Neurotoxicities in infants seen with the consumption of star anise tea. *Pediatrics* **2004**, *114*, E653–E656.
(3) Upton, R., Ed.; *Differentiation between Star Anise (Illicium verum) and the Toxic Adulterant Shikimi (Illicium anisatum)*; American Herbal Pharmacopoeia: United States, 2006.
(4) van Dijk, K. G. J. Rhabdomyolysis due to false star anise. *Neuromuscul. Disord.* **2002**, *12*, 731.
(5) Small, E. Confusion of common names for toxic and edible "star anise" (*Illicum*) species. *Econ. Bot.* **1996**, *50*, 337–339.
(6) Lane, J. F.; Koch, W. T.; Leeds, N. S.; Gorin, G. On the toxin of *Illicium anisatum*. 1. The isolation and characterization of a convulsant principle—Anisatin. *J. Am. Chem. Soc.* **1952**, *74*, 3211–3215.
(7) Schmidt, T. J.; Schmidt, H. M.; Muller, E.; Peters, W.; Fronczek, F. R.; Truesdale, A.; Fischer, N. H. New sesquiterpene lactones from *Illicium floridanum*. *J. Nat. Prod.* **1998**, *61*, 230–236.
(8) Huang, J. M.; Yang, C. S.; Kondo, M.; Nakade, K.; Takahashi, H.; Takaoka, S.; Fukuyama, Y. Merrillianin, a unique *seco*-prezizaane-type sesquiterpene, and (6*R*)-pseudomajucin from *Illicium merrillianum*. *Tetrahedron* **2002**, *58*, 6937–3941.
(9) Kakemoto, E.; Okuyama, E.; Nagata, K.; Ozoe, Y. Interaction of anisatin with rat brain γ-aminobutyric $acid_A$ receptors: Allosteric modulation by competitive antagonists. *Biochem. Pharmacol.* **1999**, *58*, 617–621.
(10) Kudo, Y.; Oka, J.; Yamada, K. Anisatin, a potent GABA antagonist, isolated from *Illicium anisatum*. *Neurosci. Lett.* **1981**, *25*, 83–88.
(11) Schmidt, T. J.; Okuyama, E.; Fronczek, F. R. The molecular structure of 2 α-hydroxyneoanisatin and structure-activity

relationships among convulsant sesquiterpenes of the *seco*-prezizaane and picrotoxane types. *Bioorg. Med. Chem.* **1999**, *7*, 2857–2865.
(12) Yang, C. S.; Hashimoto, M.; Baba, N.; Takahashi, M.; Kaneto, H.; Kawano, N.; Kouno, I. A new toxic neoanisatin derivative from the pericarps of *Illicium majus*. *Chem. Pharm. Bull.* **1990**, *38*, 291–292.
(13) Yang, C. S.; Kouno, I.; Kawano, N.; Sato, S. New anisatin-like sesquiterpene lactones from pericarps of *Illicium majus*. *Tetrahedron Lett.* **1988**, *29*, 1165–1168.
(14) Nakamura, T.; Okuyama, E.; Yamazaki, M. Neurotropic components from star anise. *Chem. Pharm. Bull.* **1996**, *44*, 1908–1914.
(15) Okuyama, E.; Nakamura, T.; Yamazaki, M. Convulsants from star anise (*Illicium verum* Hook.f.). *Chem. Pharm. Bull.* **1993**, *41*, 1670–1671.
(16) Lederer, I.; Schulzki, G.; Gross, J.; Steffen, J.-P. Combination of TLC and HPLC-MS/MS methods. Approach to a rational quality control of Chinese star anise. *J. Agric. Food Chem.* **2006**, *54*, 1970–1974.
(17) ISO 11016. *Oil of Star Anise, Chinese Type (Illicium verum Hook.f.)*; International Organisation for Standardization: Switzerland, 1999.
(18) ISO 11178. *Star Anise (Illicium verum Hook.f.)—Specification*; International Organisation for Standardization: Switzerland, 1995.
(19) Singh, G.; Maurya, S.; deLampasona, M. P.; Catalan, C. Chemical constituents, antimicrobial investigations and antioxidative potential of volatile oil and acetone extract of star anise fruits. *J. Sci. Food Agric.* **2006**, *86*, 111–121.
(20) Wichtl, M., Ed. *Herbal Drugs and Phytopharmaceuticals*; MedPharm GmbH Scientific Publishers: Stuttgart, Germany, 2004.
(21) Cook, W. B.; Howard, A. S. The essential oil of *Illicium anisatum* Linn. *Can. J. Chem.* **1966**, *44*, 2461–2464.
(22) Saltron, F.; Langella, C.; Guerere, M. Mise en évidence de contamination de badiane de Chine par d'autres espèces d'*Illicium*. *Ann. Fals. Exp. Chim.* **2001**, *94*, 397–402.
(23) Dũng, N. X.; Chính, N. D.; Leclercq, P. A. Volatile constituents of the fruit oil of *Illicium griffithii* Hook.f. et Thoms. from Vietnam. *J. Essent. Oil Res.* **1995**, *7*, 451–452.
(24) Dutta, S. C.; Saha, B. N.; Pathak, M. G.; Kanjilal, P. B.; Mathur, R. K. Essential oil of *Illicium griffithii* Hook.f. & Thoms. *J. Essent. Oil Res.* **1997**, *9*, 227–228.
(25) Tam, N. T.; An, H. L.; Bighelli, A.; Muselli, A.; Casanova, J. Advances in the chemical composition of essential oils from *Illicium griffithii* Hook.f. et Thoms. from Vietnam. *J. Essent. Oil Res.* **2005**, *17*, 79–81.
(26) Adams, R. P. *Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy*; Allured Publishing Corporation: Illinois, 2001.
(27) Ausloos, P.; Clifton, C.; Lias, S. G.; Shamim, A.; Stein, S. *NIST/EPA/NIH Mass Spectral Database*, v. 4.0; U.S. Department of Commerce: Gaitherburg, MD, 1992.
(28) Joshi, V. C.; Srinivas, P. V.; Khan, I. A. Rapid and easy identification of *Illicium verum* Hook.f. and its adulterant *Illicium anisatum* Linn. by fluorescent microscopy and gas chromatography. *J. AOAC Int.* **2005**, *88*, 703–706.
(29) Shibuya, M.; Abe, K.; Nakahashi, Y.; Kubota, S. Phenolic components from leaf oil of *Illicium anisatum* L. *Chem. Pharm. Bull.* **1978**, *26*, 2671–2673.
(30) Yakushijin, K.; Toshima, T.; Suzuki, R.; Murata, H.; Lu, S.-T. Studies on the constituents of the plants of *Illicium* species. II. Structures of phenolic components. *Chem. Pharm. Bull.* **1983**, *31*, 2879–2883.
(31) Chen, J. K.; Chen, T. T. *Chinese Medical Herbology and Pharmacology*; Art of Medicine Press, Inc.: United States, 2001.

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