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Comparison of Current Chemical and Stereochemical Tests for the Identification and Differentiation of *Pelargonium graveolens* L'Hér. (Geraniaceae) Essential Oils: Analytical Data for (-)-(1*S*, 4*R*, 5*S*)-Guaia-6,9-diene and (-)-(7*R*,10*S*)-10-epi-γ-Eudesmol

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Abstract: Commercial geranium oil samples, steam-distilled oils of authenticated plant samples, and a reference sample were investigated by GC/MS to determine the validity and applicability of a series of chemical and stereochemical tests that have been proposed in the literature to identify the country of origin, phytochemical identity or authenticity of geranium oils. The chemical tests evaluated include the ratio of the concentrations of geraniol to citronellol and the presence or absence of certain sesquiterpenes, *viz*., (-)- guaia-6,9-diene and (-)-10-epi-γ-eudesmol. The stereochemical tests include the stereochemical distribution of i) citronellol, ii) menthone and isomenthone, and iii) rose oxides. The most reliable chemical test was the presence or absence of the sesquiterpene probes. The stereochemical tests proved to be less reliable. Most of the tests could be used to classify geranium oils into general types; however, none of the tests provided a foolproof method to distinguish cultivars or country of origin. During this study, the ambiguity in the absolute stereochemistry of (-)-10-epi-γ-eudesmol and (-)-guaia-6,9-diene was addressed, and these two sesquiterpenes could serve as effective markers for the authentication of *P. graveolens* essential oils.

Keywords: Geranium oil; sesquiterpenes; chemical and stereochemical tests; *Pelargonium graveolens*; chiral GC/MS.

1. Introduction

Essential oils from plants of the *Pelargonium* L'Hér. (Geraniaceae) are commonly, but somewhat inaccurately, referred to as geranium oils. The most commercially significant of the 57 *Pelargonium* species is *P. graveolens* L'Hér [1]. The terminological confusion arises because there is a separate *Geranium* genus made up of 422 species [2]. The main applications for oils from the *Geranium* genus are for herbal medicines in the form of teas or tinctures, whereas the essential oils derived from the *Pelargonium* genus are primarily used for perfumery, cosmetics, phytotherapeutics and aromatherapy [3]. Geranium oils also play an important role in the prevention of food-borne diseases due to their antimicrobial activity [4]. In a diluted form, geranium oils can be used internally as a dietary supplement (antioxidant). Because of this multiplicity of applications, geranium oils are among the top twenty commercial essential oils [5]. The economic impact of this particular oil means that the need for quality and authenticity control is imperative. The species *P. graveolens* is particularly popular because of its rose-like aroma. The main types of commercial geranium oils are

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obtained from Reunion Island (Bourbon), North Africa (Egyptian and Moroccan) and Asia (Indian/Chinese) [6-8].

Commercial geranium oils are usually obtained by steam-distillation of the herbaceous parts of any of a wide variety of cultivars and hybrids of the *Pelargonium* genus of plants. Geranium oils are especially difficult to characterize chemically because of the complex variations observed within the natural plants. The chemical compositions of a wide variety of geranium oils have been determined primarily by gas chromatography [6-10] coupled with various types of mass spectrometric detection systems. Geranium essential oils display a wide and variable range of chemical compositions not necessarily correlated with the country of origin [11]. Many factors can influence the composition of essential oils including those involving the plant (location, age, climate, cultivars, temperature and growth regulators). Other influential factors include the sampling process (plant part, drying, comminution, distillation and storage). The most commonly cited components of geranium oils are linalool, rose oxides, menthones, citronellol, geraniol, as well as various formate, tiglate, and propionate esters of the alcohols, citronellol or geraniol. In addition, there are some purportedly unique sesquiterpene compounds, *viz.*, 10-epi-γ-eudesmol and guaia-6,9-diene, that have been suggested [5,10,12-14] as markers for distinguishing the various cultivars and hybrids of *P. graveolens*. Such identification is important in order to ensure the quality and authenticity control of commercial geranium products in an ever more competitive market environment. A secondary problem arises because the final commercial oil product may not reflect the true composition of the original plant for a variety of reasons, including user needs for a specific application, legal and market demands, and the obvious economic advantage of adulteration of natural oils with less expensive synthetic materials.

In the continuation of our efforts to improve the safety and authenticity of various phytochemicals, natural products and essential oils, an investigation of published methods for the classification of geranium essential oils was undertaken. Multiple tests to identify the plant sources for various geranium oils have been proposed. Many of these are based on the distribution of enantiomers and diastereoisomers of the optically active components of commercial geranium oils. Chiral GC columns have been used to analyze the distribution of stereoisomers with the purpose of determining the authenticity and plausible botanical source of commercial products. The stereochemistry of the oil's components is important because the odor quality of enantiomers or diastereoisomers often differ. For example, (-)-*S*-citronellol has a pleasant, rose-like odor and a peach-like flavor, whereas the (+)-*R* isomer has less desirable characteristics (bitter taste) [15]. Likewise, the (-)-*cis* enantiomer of rose oxide has better odor 'notes' and a lower odor threshold than the (+)-*cis* or either of the *trans* enantiomers [16]. Traynor [17] found that the (-) and (+) diastereoisomers of rose oxide displayed differing physiological effects, *viz.*, (+)-rose oxide had a relaxing effect while (-)-rose oxide acted as a stimulant.

1.1. Chemical Testing Methods

The ratio of the concentration of geraniol (G) to that of citronellol (C) (G:C ratio) is a clear indicator of the aroma quality and strength of geranium essential oil products. Moreover, this ratio has also been cited as a possible indicator of the type of cultivar. For example, Saxena [18] suggested that the G:C ratio of Reunion (Bourbon) oil is 1, Chinese 0.25-0.33 and a 'third type' was 0.2 although these figures are approximate. Tembe [5] made a distinction between Reunion (G:C ratio =1) and Bourbon (G:C ratio = 0.5). Egyptian type oils were suggested to have a G:C ratio of 0.25.

Other authors [5,10,11] have suggested that the presence or absence of the sesquiterpenes 10-epi-γ-eudesmol and guaia-6,9-diene in *Pelargonium* oil is a clear indicator of the cultivar from which the essential oil was derived or the country of origin. In particular, 10-epi-γ-eudesmol is found in oils from Africa and Egypt; whereas, the guaia-6,9-diene is prominent in oils from Reunion (Bourbon) and China. The International Organization for Standardization (ISO 11024-1) specifies that guaia-6,9-diene is present in Bourbon geranium oil while 10-epi-γ-eudesmol is absent in Bourbon but present in African geranium oil.

The exact structure and stereochemistry of these two sesquiterpenes are critical but difficult to determine because of the multitude of very similar sesquiterpenes found in natural plants such as *P. graveolens*. The usual identification methods include chromatographic retention indices or times, mass spectra, and MS library searches. However, all of these analytical methods are problematic with

sesquiterpenes. A thorough literature survey suggested that there was some ambiguity associated with the optical rotation of 10-epi-γ-eudesmol [19,20] and the absolute stereochemistry of guaia-6,9-diene [13]. Isolation, NMR characterization and optical rotation measurements are needed for the unambiguous determination of the exact structure and stereochemistry of sesquiterpenes to be used as probes for the chemical testing methods.

1.2. Stereochemical Test Methods

The stereochemistry of natural products is often unique because of the biochemical processes involved in the generation of secondary metabolites. The aroma quality, flavor, toxicity and biological activity of oils often depend upon the stereochemistry of the components. Thus, analysis of the chirality of certain components of *Pelargonium* oils could possibly serve as a more subtle mechanism for distinguishing the type, authenticity and country of origin of commercial oils. For example, Ravid [15] measured the enantiomeric composition of citronellol in prepared and commercial geranium oils. It was observed that in most cases, the citronellol appeared as a mixture; however, the (-)-*S* enantiomer was often predominant with a range of 50-80 % for geranium oils.

Other researchers [21-23] analyzed geranium essential oils for the enantiomers of isomenthone and menthone. In plant and commercial geranium oils, these groups found only (+)-(1*S*,4*R*) menthone and (-)-(1*S*,4*S*) isomenthone with high enantiomeric purity.

The authenticity of geranium oils can also purportedly be determined from the distribution of stereoisomers of the rose oxides. In natural geranium plants, (-)-diastereoisomers of *cis* and *trans*-rose oxides derived from (-)-(4*S*) citronellol are usually dominant [22,24]. Conversely, when analyzing 18 geranium oils, Doimo reported, the presence of *cis* and *trans* (+)-rose oxide diastereoisomers [25] with little or no (-) diastereoisomers.

The objects of the current investigation were to compare and evaluate the various chemical and stereochemical tests proposed in the literature to determine the type and authenticity of various forms of geranium oils. Evaluation of the proposed tests was carried out with a limited set of samples of commercial oils, authenticated plants, and a reference standard to determine the validity and applicability of each comparative test.

2. Materials and Methods

2.1. Samples

Ten commercial geranium oil samples, steam distilled oils of three authenticated plant samples, and a reference sample were investigated. Samples S1-S10 were commercial geranium oils purchased online. The labeled sources were Egypt (S1, S4, and S8), France (S3, S5 and S7), China (S2), South Africa (S9) and China/France/Morocco (S6). One commercial sample (S10) was of unknown origin. The details of the steam distillation process for the commercial samples were not available. The oil samples S14 and S15 were obtained from the steam distillation of the authentic plant samples provided by Indian Institute of Integrative Medicine (IIIM). The plant materials were cultivated at the field station in Bonera (Pulwama, India). Taxonomic authentication was provided by Dr. Y.S. Bedi. In addition, another plant sample was obtained from the Maynard W Quimby Medicinal Plant Garden at the University of Mississippi. The original plants were purchased from the Richters Herbs, Goodwood, ON, Canada and authenticated by taxonomist, Dr. Aruna Weerasooriya. The samples are available in the living collection (Accession number: AGC-052-05-11) and a herbarium voucher also deposited at the Maynard W. Quimby Medicinal Plant Garden at the University of Mississippi. The harvested plant was washed and dried for 24 hours at room temperature. About 500 g of leaves were used to produce the essential oil (Plant), and the distillation was conducted in a Clevenger-type glass apparatus for 4 hours. The yield of oil from the leaves was 0.16% by weight. One sample (Sigma) was purchased from Sigma Aldrich (#W250813). The country of origin was China as per the label and the details of the steam distillation process were not known.

2.2. Instrumentation

Agilent 7890 gas chromatograph coupled with an Agilent 5975 single quadrupole mass specific detector with an electron impact source was used for all of the analyses. Helium was used as the carrier gas. The injector and MS source temperatures were 250 °C and 230 °C, respectively. The MS instrument was operated in the scan mode over a range of 30-550 Daltons with an electron impact source operating at 70 eV. The GC/MS library was the NIST/Wiley mass spectral library.

2.3. Chromatographic columns

Standard Column: Agilent DB-5 [(5%-phenyl)-methylpolysiloxane] column (30 m x 0.25 mm x 25 µm). Temperature program: 50 °C for 4 min, 2 °C /min to 160 °C, 8 °C /min to 250 °C, hold for 20 min.
Chiral Column: Restek Rt-βDEXsa (Alkylated β-cyclodextrins in cyanopropyl-dimethylpolysiloxane) column (30 m x 0.25 mm x 25 µm). Temperature program: Start at 40 °C, 1 °C/min to 120 °C, 5 °C/min to 160 °C. The split ratio was 25:1, and the linear velocity was 80 cm/sec.

2.4. Authentication of Marker Compounds

Compounds 1-5, 7-10 and 12 were purchased from Sigma-Aldrich (St. Louis, MO). Isomenthone (6) was purchased from Erdogmus Parfum Sanay (Istanbul, Turkey). Compounds 13 and 15, *viz*., (-)-guaia-6,9-diene and (-)-10-epi-γ-eudesmol, were not commercially available and were isolated in-house. Compounds 11, 14 and 16 were all identified by the comparison of the spectra with the databases (Wiley and NIST) and the chromatographic retention indices. The enantiomeric standards were all purchased from Sigma-Aldrich (St. Louis, MO), and the purities were greater than 98.0%.

2.4.1. (-)-(1S, 4R, 5S)-Guaia-6,9-diene **(13)**

Identification of (-)-(1*S*, 4*R*, 5*S*)-guaia-6,9-diene was performed by 1D and 2D NMR experiments. $[\alpha]_D^{20}$= -41.6 (c= 1.5 g/100mL in EtOH on a sample found by GC to be 80% pure). ^{1}H NMR (500 MHz, C_6D_6) δ 5.49 (ddt, *J* = 8.7, 3.3, 1.6 Hz, 1H), 5.34 (dd, *J* = 4.5, 1.6 Hz, 1H), 3.03 (ddt, *J* = 15.6, 3.6, 1.6 Hz, 1H), 2.90 (q, *J* = 4.6 Hz, 1H), 2.24 (heptet, *J* = 6.8 Hz, 1H), 2.22-2.18 (m, 1H), 2.16 (ddd, *J* = 15.6, 8.7, 1.6 Hz, 1H), 1.88 (ddq, *J* = 8.7, 6.7, 1.9 Hz, 1H), 1.81 (dp, *J* = 12.9, 4.6 Hz, 1H), 1.67 (ddt, *J* = 12.9, 9.0, 4.2 Hz, 1H), 1.60 (s, 3H), 1.47 (qd, *J* = 11.9, 4.7 Hz, 1H), 1.28 (qd, *J* = 11.9, 4.7 Hz, 1H), 1.10 (d, *J* = 6.8 Hz, 3H), 1.01 (d, *J* = 6.7 Hz, 3H), 0.99 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (126 MHz, C_6D_6) δ 152.7, 138.7, 121.0, 118.9, 49.0, 44.1, 38.8, 36.8, 29.6, 29.2, 27.9, 24.9, 21.5, 21.4, 16.1. Mass spectral peaks were observed at masses characteristic of sesquiterpene hydrocarbons, *viz*., *m/z* 204, 189, 161, 135, 119, 105 and 91. These values agreed with literature data [10].

2.4.2. (-)-10-epi-γ-Eudesmol **(15)**

Identification of (-)-10-epi-γ-eudesmol was performed by 1D and 2D NMR. $[\alpha]_D^{20}$= -49.0 (c= 0.6 g/100mL in $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.70 (bm, 1H), 2.10 (bm, 1H), 1.89 (bm, 2H), 1.68 (bm. 2H), 1.68 (bs, 3H), 1.67 (m, 1H), 1.66 (m, 1H), 1.58 (m, 1H), 1.51 (m, 1H), 1.39 (m, 1H), 1.32 (m, 1H), 1.28 (m, 1H), 1.24 (s, 3H), 1.18 (s, 3H), 1.08 (s, 3H).^{13}C NMR (100 MHz, $CDCl_3$) δ 135.1, 126.1, 74.7, 44.2, 39.6, 38.2, 34.6, 32.9, 30.0, 28.0, 26.0, 25.5, 22.7, 19.8, 19.0. Mass spectral peaks were observed at *m/z* 222, 204, 189,161, 133, 91 and 59. The NMR data were in agreement with the literature data [26,27].

The experimental procedures for the isolation of these two sesquiterpenes are given in the supplemental material.

3. Results and Discussion

Sixty-four components comprised 97.84% of the total oil of sample S5. The list of the major compounds along with the relative retention indices is given in Table 1. Compound identification involved the comparison of the spectra with the databases (Wiley and NIST) using a probability-based matching algorithm. Further identification was based on the relative retention indices (RRI) compared with literature [28-30] and the standard references isolated in-house or purchased from commercial

sources. Figure 1 shows a typical total ion gas chromatogram of a commercial *Pelargonium* (Geranium) essential oil along with the structures of several common compounds found in geranium oils. The identity and structure of the major components are given, and the individual components proposed as markers are shown in bold text. Two internal standards, *viz*., *n*-decane and *n*-dodecane, were added to each sample. All measured peak areas were normalized to *n*-decane.

Table 1. Chemical Composition of *Pelargonium graveolens* Essential Oil (S5)

RRI[a]	Compounds	Peak Area (%)
947	α-pinene	0.36
993	myrcene	0.16
1026	limonene	0.21
1067	*cis*-linalool oxide (*Furanoid*)	0.22
1100	linalool	3.50
1107	*trans*-rose oxide	1.56
1121	*cis*-rose oxide	0.57
1152	menthone	1.79
1162	isomenthone	5.63
1185	menthol	0.15
1194	α-terpineol	0.34
1238	β-citronellol	32.09
1241	β-citral	0.48
1259	geraniol	8.20
1265	myrtanol	0.22
1272	α-citral	0.36
1277	citronellyl formate	9.99
1300	geranyl formate	2.28
1339	MW 204	0.17
1351	citronellyl acetate	0.39
1365	α-cubebene	0.58
1372	β-bourbonene	1.40
1379	geranyl acetate	0.30
1381	β-elemene	0.21
1406	caryophyllene	1.51
1416	β- cubebene	0.11
1423	α-guaiene	0.36
1429	guaia-6,9-diene	4.08
1435	isoledene	0.57
1437	citronellyl propionate	0.83
1440	humulene	0.38
1443	aromandendrene	0.29
1457	β-cadinene	0.33
1461	γ-muurolene	0.13
1464	germacrene D	2.23
1471	β-selinene	0.31
1473	ledene	0.82
1477	γ-elemene	0.38
1483	α- muurolene	0.32
1495	γ-cadinene	0.33
1501	δ-cadinene	1.27
1504	calamenene	0.61
1515	citronellyl butyrate	1.52
1523	alloaromadendrene oxide	0.20
1542	geranyl butyrate	1.12
1551	spathulenol	0.29
1554	caryophyllene oxide	0.23
1557	MW 234	0.41
1564	phenethyl tiglate	0.98
1577	geranyl propionate	0.30
1586	cubenol	0.26

1590	10-epi-γ-eudesmol	2.14
1596	MW 222	0.14
1598	2,6-octadiene, 2,6-dimethyl-	0.16
1603	guaiol	0.12
1608	cubedol	0.12
1611	β-guaiene	0.21
1618	β-eudesmol	0.31
1620	globulol	0.29
1625	MW 238	0.17
1632	citronellyl tiglate	0.80
1649	citronellyl butyrate	0.22
1661	geranyl tiglate	1.58
1675	nerol acetate	0.25
	Total	**97.84**

[a] RRI: Relative retention index calculated on the DB-5 column relative to C_8-C_{28} *n*-alkanes

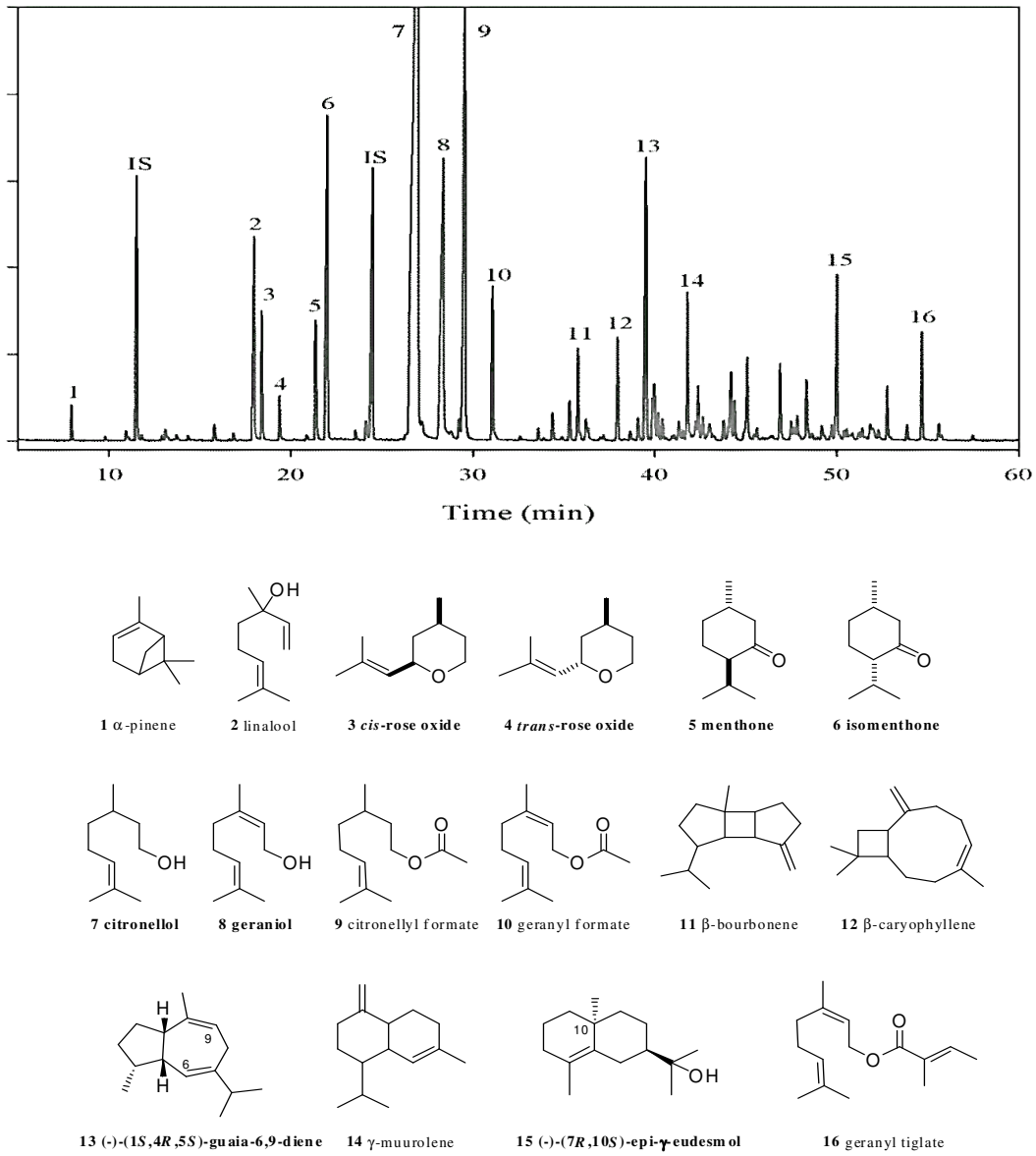

Figure 1. Typical Total Ion Gas Chromatogram for a Commercial Geranium Oil Product (Sample S5) along with the Structures of Some Typical Components. IS = Internal Standard

3.1.Chemical Tests for Geranium Samples

3.1.1 Ratio of the Concentrations of Geraniol to Citronellol (G: C Ratio)

The G:C ratios for all of the essential oil and plant samples are shown in Figure 2 along with the ratio limits previously suggested for the different types of geranium oils [5,18]. In this case, the commercial samples with reported origins in Egypt and China have similar ratios of *ca*. 0.4, which are higher than the suggested ratios. Whereas the samples from France (Bourbon) displayed ratios in the range 0.14 – 0.26, *i.e*., less than that the proposed ratios for Bourbon type oils. The sample of unknown or multiple origin had a ratio of 0.4 and were thus probably from China. The authenticated plant samples from India showed high ratios while those from China and the USA displayed low ratios of *ca*. 0.2. No satisfactory pattern was discerned in the G:C ratio data to justify this test as a method to distinguish the type or origins of the investigated oils. This observation is in agreement with early work by Lis-Balchin [11] who found that the G:C ratios for Bourbon oils varied from 0.15-0.56, Chinese oils varied from 0.09-0.56, while Egyptian and Moroccan geranium oils displayed G:C ratios from 0.45-0.59. The author's conclusion was that there was no direct correlation between the chemical compositions of geranium oils and their geographical source. *Thus, it would be very difficult to ascertain the type of cultivars or countries of origin for the limited set of geranium oils investigated herein based solely on G:C ratios.*

3.1.2. Presence of (-)-10-epi-γ-Eudesmol or (-)-Guaia-6,9-diene Sesquiterpene Markers

Both of these sesquiterpenes are considered to be detrimental to the olfactory quality of geranium oils [31,32]; however, the popularity of these two probes for the characterization of geranium oils requires an in-depth study. The relative concentrations of these two markers for each of the samples are given in Table 2 along with the G:C ratios. The concentrations are expressed as peak areas relative to the dodecane internal standard which was present at the same concentration in all of the samples. The samples from Egypt, China, South Africa, and India generally contained only (-)-(10)-epi-γ-eudesmol (**15**) with little or no (-)-guaia-6,9-diene (**13**). The sample from the USA (Plant) as well as the oil samples from France (S3, S5, S6, & S7) all contained significant amounts of **13** with little or no **15**. Sample S5, however, contained significant quantities of both markers. The 'Sigma' sample reported to originate from China contained (-)-guaia-6,9-diene with no (-)-10-epi-γ-eudesmol in contrast to sample S2.

Table 2. Geraniol:Citronellol Ratios and Concentrations of Sesquiterpenes from Relative Peak Areas

Sample	Purported Country of Origin	(-)-10-epi-γ-eudesmol	(-)-guaia-6,9-diene	G:C Ratio
S9	South Africa	5.2		0.36
S4	Egypt	5.2		0.42
S10		5.5		0.44
S2	China	5.7		0.38
S8	Egypt	5.9		0.39
S1	Egypt	6.2		0.41
S15	India	6.4		0.73
S14	India	6.8		0.52
S7	France		6.0	0.14
S3	France		6.3	0.15
S6	China/France/Morocco		6.5	0.17
Sigma	China		7.8	0.18
Plant	USA		8.7	0.18
S5	France	2.5	4.1	0.26

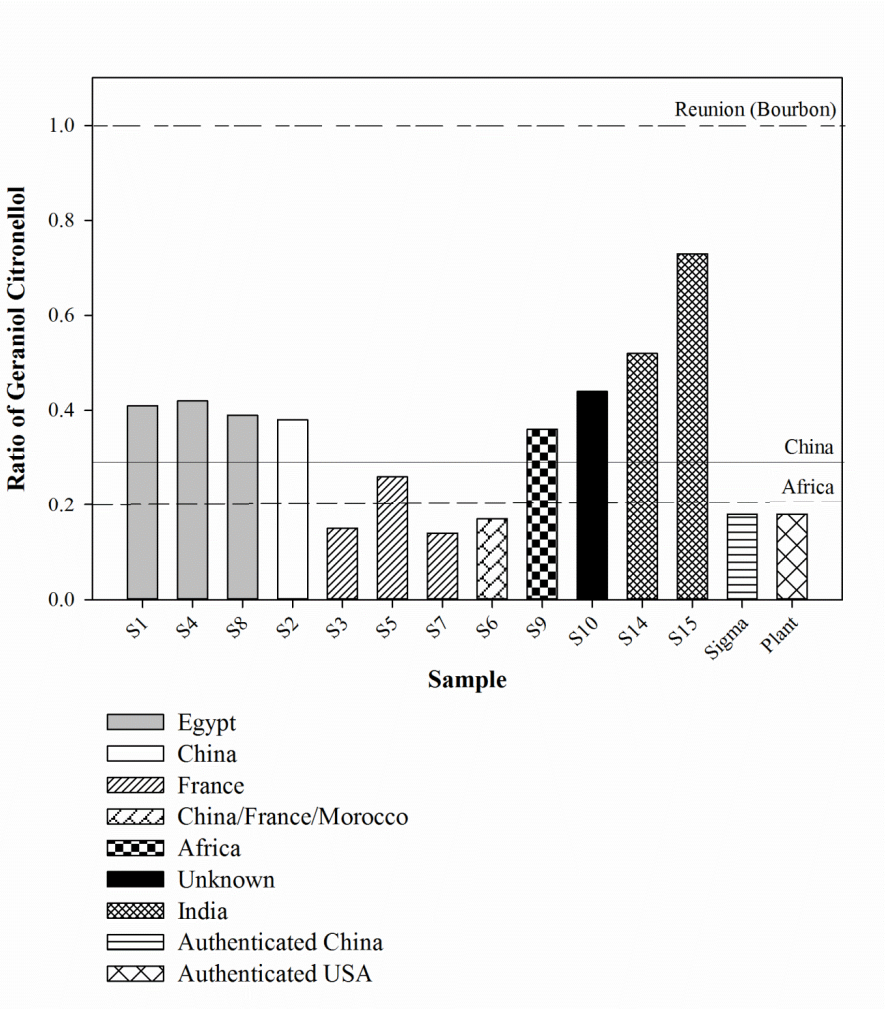

Figure 2. Experimental G:C Ratio for the Commercial and Authenticated Plant Oils

In this limited data set it was observed that if the observed G:C ratio was ≤ 0.25 (citronellol concentration was at least 4 times higher than that of geraniol), only (-)-guaia-6,9-diene was found to be the marker sesquiterpene; whereas, only (-)-10-epi-γ-eudesmol was found to be present in oils with a G:C ratio >0.25. Remarkably, sample S5, which contained both sesquiterpenes, had a measured G:C ratio of 0.25.

Unfortunately, the observed correlation between the G:C ratio and the sesquiterpene probes does not hold for larger data sets. For example, Lis-Balchin [11] carried out the same type of investigation and found that 13 of 16 samples of Bourbon or Chinese Geranium oils contained (-)-guaia-6,9-diene with no (-)-10-epi-γ-eudesmol. Only nine of 13 Egyptian and Moroccan samples contained just (-)-10-epi-γ-eudesmol. One Moroccan sample contained both the eudesmol and guaiadiene markers. The correlation with G:C ratio was not evident because for samples with G:C > 0.25, 11 contained the eudesmol probe while 13 contained (-)-guaia-6,9-diene. Thus, in both studies, a general trend was observed albeit with significant exceptions. The presence of **15** or **13** is the best chemical test to characterize geranium oils; however, the probability of exceptions must be taken into account.

Because of the significance of this particular test involving sesquiterpenes, the two probe materials were isolated and investigated further by NMR and polarimetry. Compound **13** was determined to be (-)-(1*S*, 4*R*, 5*S*)-guaia-6,9-diene. Likewise, compound **15** was determined to be (-)-(7*R*,10*S*)-10-epi-γ-eudesmol. The mass spectra as well as 1D and 2D NMR spectra along with HMBC, NOESY, DQFCOSY, and HMQC correlations are given in the supplementary material.

3.2. Stereochemical Tests for Geranium Samples

Figure 3 shows a total ion chromatogram for a series of standards of the typical chiral components observed in geranium essential oils. The enantiomers of all of the standards were well resolved except for citronellol.

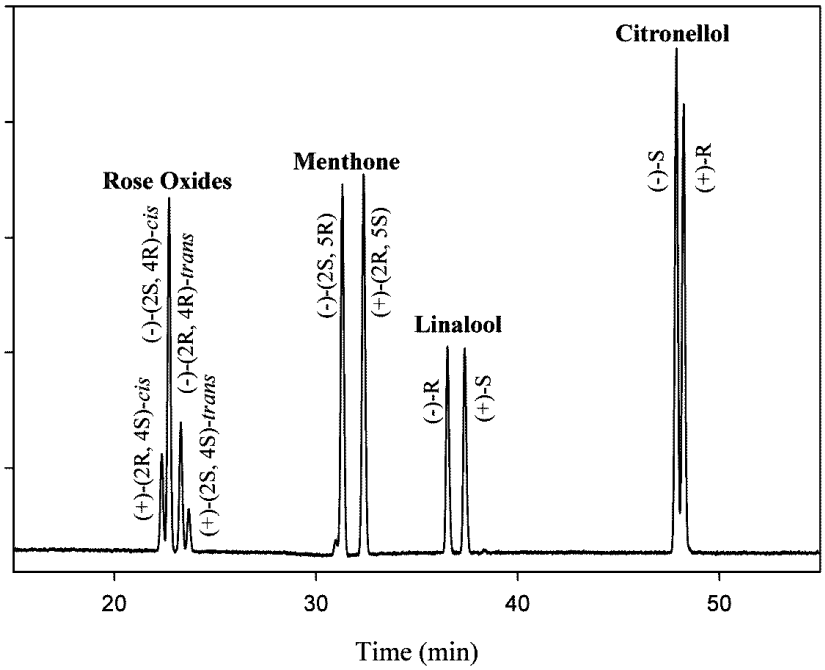

Figure 3. Gas Chromatogram of Chiral Standard Materials

3.2.1. Citronellol Enantiomers

The results for the analysis of (-)-*S* and (+)-*R* citronellol in the samples showed that the ratio of (-)/(+) isomers ranged from 1.3 – 1.9 with little systematic variation. In every case, the (-)-*S* isomer was dominant.

The literature on the significance of the enantiomeric ratio of citronellol in geranium oils is also contradictory. Ravid [15] found that the ratio of (-) to (+) was in the range 1-3.5 with no oils showing an excess of the *R*-(+)-isomer. Commercial sources [23] suggest that the (-)-*S* isomer is always dominant in authentic oils samples and that the appearance of racemates is indicative of adulteration of the natural product. On the other hand, Kries [22] found the (+) to (–) ratio of enantiomers in geranium oils varied from 0.83 to 4.0. More recently, Doimo [25] found that the (+)-*R* isomer was always dominant in a wide variety of geranium oils from China, Reunion, Morocco, Egypt and Australia. However, this observation was based on the indirect calculation of the enantiomeric distribution of citronellol, which was chromatographically unresolved, from the stereoisomeric distribution of rose oxides. In summary, the enantiomeric distribution of citronellol is not a clear marker for the origin, biology, or authenticity of geranium oil samples although most geranium oils contain the (-)-*S* enantiomer as the dominant component. The presence of the (+)-*R* isomer or a racemic mixture may indicate adulteration.

3.2.2. Menthone and Isomenthone

Naturally occurring (-)- isomenthone and (+)-menthone are diastereoisomers that are common components of most geranium oils. These diastereoisomers can be separated on a non-chiral GC column (Figure 1). The distribution of these diastereoisomeric compounds may provide a method to distinguish synthetic analogs from natural products. The distribution of (-)-(2*S*, 5*S*) isomenthone and (+)-(2*R*,5*S*) menthone were determine, and the (-)/(+) ratio varied from 1.5 - 100. In every sample, (-

)-isomenthone and (+)-menthone were present with isomenthone dominant. No adulteration with synthetic, racemic analogs was detected. Again, however, the complete absence of (+)-isomenthone and (-)-menthone is not universal. For example, Doimo [25] and Kreis [22] found several geranium oils that contained both menthone and isomenthone enantiomers.

3.2.3. Rose Oxides

The stereoisomers rose oxide present a unique set of potential markers because of the close chemical relationship between rose oxide and citronellol. Citronellol in geranium oils may be enzymatically oxidized to rose oxide [24]. It has been hypothesized [33] that the stereochemical distribution of the rose oxide enantiomers and diastereoisomers should reflect the enantiomeric distribution of the precursor citronellol in unadulterated geranium oils. That is, if (-)-*S* citronellol is dominant in the oil sample, then the (-)-*cis* and (-)-*trans* rose oxides should also be dominant [34]. Doimo [25] used this presumed relationship to determine the enantiomeric distribution of citronellol that could not be resolved chromatographically from the (+)/(-) distribution of the easily resolved rose oxide stereoisomers.

Thus, stereochemical analysis of the rose oxide isomers may provide a chiral fingerprinting method to differentiate geranium oils. In the present study, a chiral analysis of both rose oxide and citronellol in each sample was carried out. The resolution of the citronellol enantiomers was not complete [23]; however, the resolution was sufficient to allow a reasonable estimate of the enantiomeric ratio. The absolute abundance of the citronellol and rose oxide stereoisomers varied dramatically (by a factor of 30) within the sample set. However, the percentage of a given pair of (+) diastereoisomers of rose oxide could be correlated with the percentage the (+) citronellol enantiomer in any given sample. The correlation can be presented as a plot of the ratio of the sums of the peak areas $\frac{\sum(-)Rose\ Oxides}{\sum(+)Rose\ Oxides}$ vs. the ratio of the peak areas for the citronellol enantiomers, *i.e.*, $\frac{(-)Citronellol}{(+)Citronellol}$. The results of this analysis are shown in Figure 4. The average of the ratios was 0.73 ($\pm$ 0.04). If all of the rose oxides in the sample were produced by the oxidation of citronellol, the slope of the plot would be unity. The experimental data indicate that a racemic mixture of citronellol would produce only 75% of the theoretical amount of (-) rose oxides compared to the (+) rose oxides. This may be due to the fact that (-) citronellol can be converted to (-) isomenthone and (+) menthone as well as (-) rose oxides. The analogous conversion does not occur for (+) citronellol in the given *P. graveolens* samples.

Thus, any attempt to identify adulteration of geranium oils by analysis of rose oxides is questionable at best. Moreover, there is no clear pattern to allow the identification of a particular type of oil solely from the distribution of either rose oxide or citronellol stereoisomers.

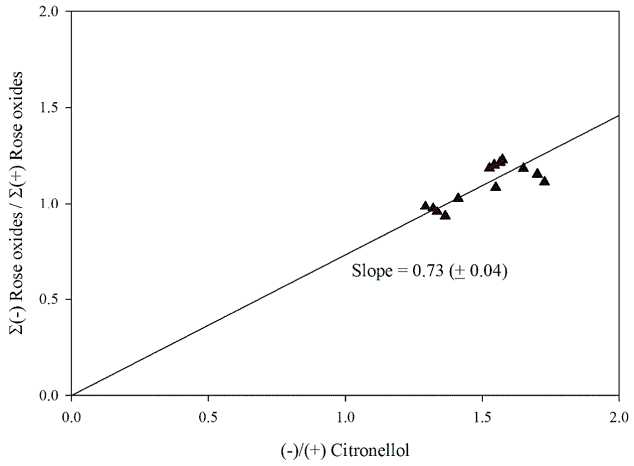

Figure 4. Plot of $\frac{\sum(-)Rose\ Oxides}{\sum(+)Rose\ Oxides}$ versus $\frac{(-)Citronellol}{(+)Citronellol}$

4. Conclusion

Of the chemical tests, the presence of the sesquiterpenes probes appears to be the most reliable. In the present data set, all of the samples from Egypt, India, and S. Africa contained (-)-10-epi-γeudesmol but not (-)-guaia-6,9-diene. The samples from France or the USA contained the diene but no eudesmol. One Chinese sample contained (-)-10-epi-γ-eudesmol while the other contained (-)-guaia-6,9-diene. However, in the more extensive sample studied by Lis-Balchin [11], 6 of 7 Bourbon samples contained (-)-guaia-6,9-diene; the same was true for 8 of 10 Chinese samples; 8 of 11 Egyptian samples contained 10-epi-γ-eudesmol; and one Moroccan sample contained the eudesmol sesquiterpene only while the other contained <1% of both sesquiterpenes. Thus, the trend is clear; however, the possibility of exceptions must be taken into account.

Among, the stereochemical tests, neither of the three tests, viz., citronellol, isomenthone/menthone, or rose oxides, provides a foolproof method to distinguish between cultivars, hybrids, or countries of origin of commercial geranium oils. In conclusion, the application of chemical or stereochemical tests to identify the provenance, authenticity, or geographical source of geranium oils is limited in scope. This is due to the very complex chemical composition of the plants and the wide variety of factors that can influence the chemical composition. No single chemical test was found to be infallible for the characterization of geranium oils. Authenticated plant samples often produced discernible patterns in the chemical or stereochemical distribution of chemical components. The reverse was, unfortunately not true. That is, it was not generally possible to identify a given geranium plant from patterns of the chemical composition of the essential oil of that plant. Whether or not this dilemma is unique to geranium plants and oils is an open question.

In the current investigation, the chemical and stereochemical tests were evaluated individually. A more holistic approach may be necessary. That is, in order to identify the authenticity and provenance of commercially important geranium oils, it may be necessary to apply more than one test at a time. For example, the possible correlation between the G:C ratio and the presence of sesquiterpenes discussed previously. Another possible correlation was observed between citronellol, iso/menthone, and the stereoisomers of rose oxide. Regarding the enantiopurity of citronellol, the majority of the samples in our dataset contain slightly higher amount of (-)-citronellol than the (+) isomer. Likewise, the metabolites (-)-isomenthone, (+)-menthone, (-)-*cis* rose oxide and (-)-*trans* rose oxide enantiomers presumably derived from (-)-S-citronellol were found to be the major oxidative metabolites observed in the oils. This prevalence may be due to the preferential oxidation of *S*-(-)-citronellol by metabolic enzymes very specific to *P. graveolens*. Molecular studies of the biogenic origin of these oxidative metabolites along with identification of the responsible monoterpene synthases may provide crucial information regarding substrate specificity in *Pelargonium* species.

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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