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Isomeric Enhancement of Davanone from Natural Davana Oil Aided by Supercritical Carbon Dioxide

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The chemical nature of davanone isolated from natural davana oil via packed column preparative supercritical fluid chromatography with a carbon dioxide-based mobile phase has been defined. Analyses used to characterize davanone included nuclear magnetic resonance spectroscopy, optical rotation, mass spectrometry, headspace solid-phase microextraction, enantiomeric purity via gas chromatography (GC), and GC-coupled olfactometry. For comparison, natural davana oil was subjected to the same types of measurements. The enriched davanone sample was nearly 100% optically pure. This indicates that fractionation of the davana oil with supercritical fluids at near room temperature had little effect on the optical integrity of the sample.

KEYWORDS: Chiral chromatography; davana oil; davanone; olfactometry; supercritical fluid extraction

INTRODUCTION

The oil of davana, which initially was of importance in the perfume industry, has been the subject of several investigations that have had as their goal the isolation and characterization of the volatile components of the oil (*1*−*5*). Davana is an aromatic herb, cultivated in India. The oil of davana is produced from dried davana plants by steam distillation with yields of approximately 0.2%. The oil is a brownish or dark greenish viscous liquid, very aromatic, with a persistent odor somewhat reminiscent of bourbon. Optical rotation values ranging from +35° to +5° have been reported, which most probably depend on the purity of the oil. Davanone has been shown to be the main component of the davana essential oil derived from *Artemisia pallens*, varying between 30 and 60% by weight (*3*, *6*). Davanone has also been determined to be the main ingredient of the essential oil derived from two other *Artemisia* species (*7*−*9*). Natural sesquiterpene (+)-davanone has been assigned the structure shown in **Figure 1A** on the basis of spectroscopic and degradative evidence and is referred to as 6*S*,7*S*,10*R*-2,6,-10-oxydododeca-2,11-dien-5-one, the cis/threo isomer.

Synthetic confirmation of this structure was achieved many years ago, but the route that was followed allowed no steric control, so that the synthetic material yielded a stereoisomeric mixture of all four possible (±)-diastereoisomers (*10*, *11*). The similarity of the observed and published spectra provided clear confirmation of the proposed gross structure of davanone. A base-catalyzed equilibrium mixture of natural davanone in *tert*-butyl alcohol has been shown to yield four isomeric forms within 30 min in proportions (by mole) of about 12:7:30:50, with the natural davanone corresponding to the major isomer (*3*).

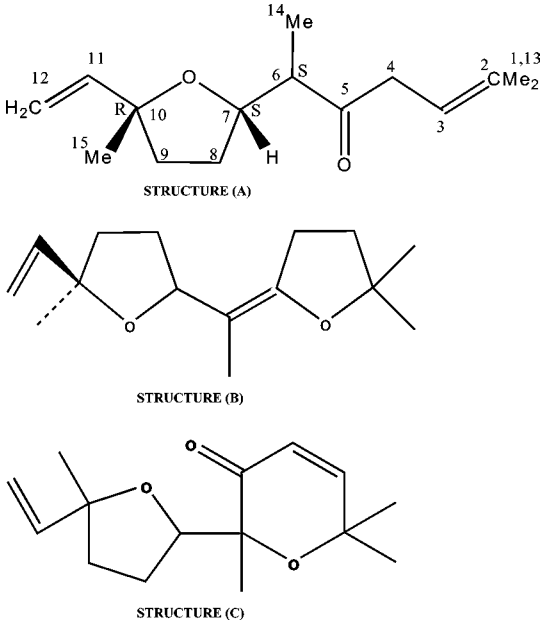

Figure 1. Molecular structure of davanone.

Davanone has been reported to be odorless (*1*, *6*). Therefore, the odoriferous constituents of the oil have been reported to be other components such as davana ether. Interest nevertheless continually arises as to the contribution of davanone to the sensory attributes of the product because several literature references have suggested the absence of any odor associated

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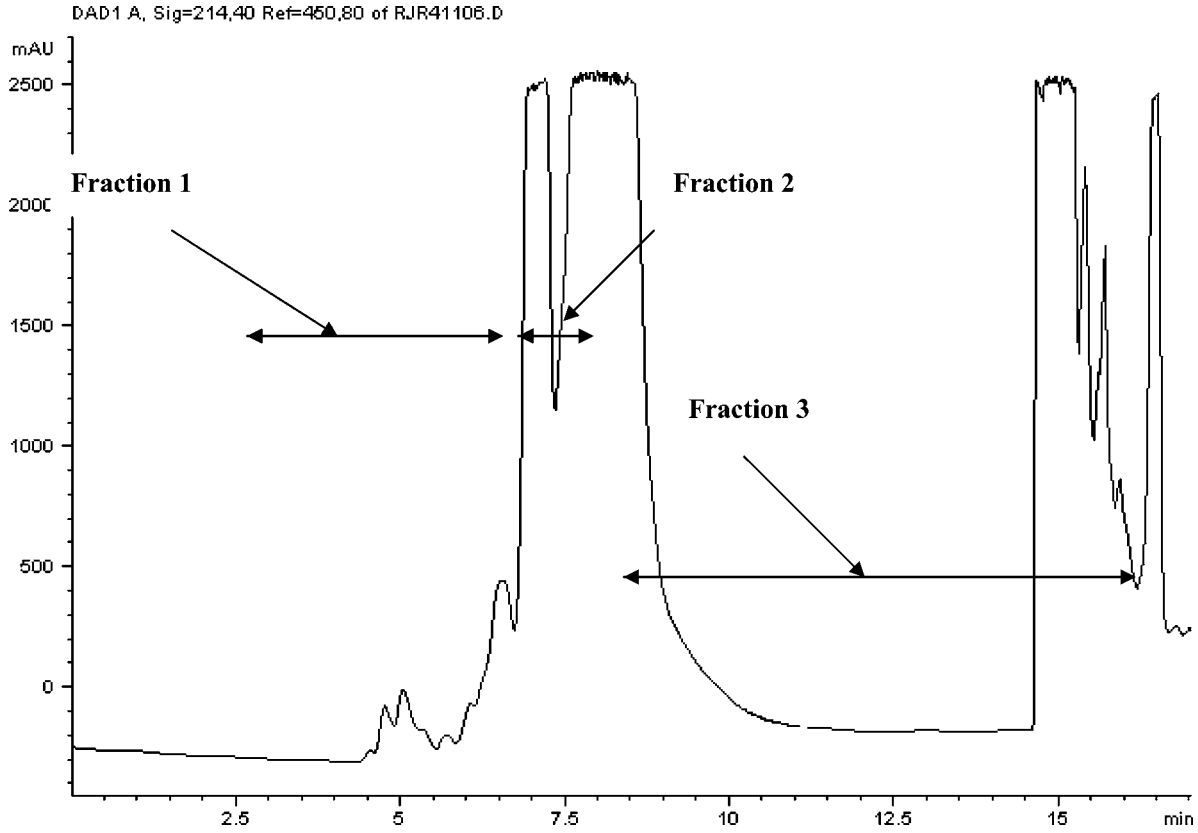

Figure 2. Fractionation of natural davana oil via preparative SFC with collected fractions noted.

with davanone. This paper therefore deals with the isolation and identification of the components of natural davana oil.

Fractional distillation in our laboratory for the isolation of davanone has been explored but with unfavorable results. On the other hand, the separation and purification of natural davana oil using carbon dioxide-based supercritical fluid extraction (SFE) and chromatography (SFC) have been successful; these approaches in the past have been effectively employed in the isolation and purification of a wide variety of natural products (*12*−*14*). The headspace analysis of nonfractionated natural davana oil using solid-phase microextraction (SPME) is also reported. SPME affords one the ability to detect, via an enrichment process, the presence of relatively low molecular weight, volatile compounds that otherwise would most likely be masked by accompanying solvent should solutions of the material be prepared for analysis. On some occasions relatively low molecular weight compounds can have significant olfactory sensory attributes at low concentrations (*15*−*17*). In this regard, gas chromatography/olfactometry (GC/O) has become a rather routine approach for the analysis of aromas and flavors (*18*). SPME in combination with GC/O and gas chromatography/mass spectrometry (GC/MS) has been reported for the characterization of aroma compounds in a number of matrices (*19*−*21*). Consequently, we also report here, alongside our SFE/SFC results, the application of SPME/GC/O/MS to davana oil in hopes of providing detailed characterization of the components present therein.

MATERIALS AND METHODS

Instrumentation. Commercial davana oil was obtained from Charabot (Grasse, France). The SPME DVB/Carboxen/PDMS fiber (2 cm, 50/30 μm) was purchased from Supelco, Bellefonte, PA. An Agilent GC 6890 and MS 5973 were employed for GC/MS measurements. An achiral DB-WAXetr open tubular column (30 meters, 0.25 mm i.d., 0.25 μm film thickness) was obtained from J&W Scientific. Four chiral stationary phases were purchased from Restek Corp. (Bellefonte, PA). Each phase incorporated various combinations of alkylated-β-cyclodextrins into a cyanopropyl-dimethylpolysiloxane liquid stationary phase. The columns were each 30 m long, 0.25 mm i.d., 0.25 μm film thickness and included (1) RT-β-dexse, (2) RT-β-dexsp, (3) RT-β-dexsm, and (4) RT-β-dexcst. The olfactometer was obtained from Microanalytics (Round Rock, TX) and customized such that the split ratio between the mass spectrometer and olfactometer was 10:90 rather than 50:50. All packed column SFC separations were obtained using a Mettler-Toledo (Wilmington, DE) Mini-gram Berger SFC system. Analytical-scale, silica-based stationary phases were cyano, diol, silica, propylamino, and octadecyl from Supelco Inc.; Deltabond cyano from Keystone Scientific, Bellefonte, PA; and 2-ethylpyridine from Princeton Chromatography Inc., Princeton, NJ. Carbon NMR spectra were obtained on a JEOL Ltd. (Tokyo, Japan) Eclipse 500 MHz instrument with a 45° pulse and 1 s relaxation delay (2276 scans). Proton NMR spectra were obtained on a Varian Inc. (Palo Alto, CA) INOVA 400 Mz instrument.

SPME-GC-MS. One gram (±0.05 g) samples of davana oil in triplicate were placed in screw-cap-sealed 20 mL glass sampling vials. These samples were allowed to equilibrate overnight under ambient laboratory conditions prior to analysis. The SPME fiber was conditioned per the manufacturer's directions. SPME/headspace/GC/MS operating conditions for natural davana oil were as follows: injection port temperature, 250 °C; injection, splitless; column flow, constant, 1 mL/min He; column oven initial temperature, 30 °C; column oven initial time, 1 min; column oven initial ramp rate, 10 °C/min; column oven final temperature, 250 °C; SPME exposure time, 10 min; SPME exposure temperature, 35 °C; SPME desorption time, 1 min; mass spectrometer transfer line temperature, 250 °C; mass spectral databases, NBS, Wiley; mass spectrometer configuration, electron impact, 70 eV.

GC/MS/O. A methylene chloride solution of natural davana oil was prepared at approximately 5.0 mg/mL. GC/MS/O operating conditions were as follows: injection port temperature, 250 °C; injection, 2 μL, splitless; column flow, constant, 1 mL/min He; column oven initial temperature, 60 °C; column oven initial time, 1 min; column oven initial

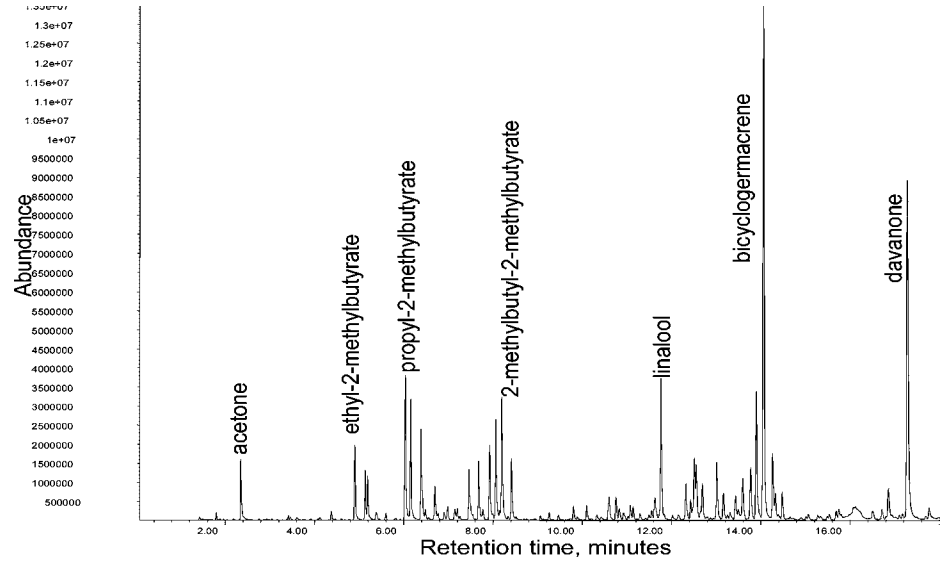

Figure 3. Total ion chromatogram of headspace above natural davana oil using SPME/GC/MS.

ramp rate, 5 °C/min; column oven final temperature, 250 °C; mass spectrometer transfer line temperature, 250 °C; mass spectral databases, NBS, Wiley; mass spectrometer configuration, electron impact, 70 eV; MS/sniff port split ratio, 10:90; sniff port transfer line temperature, 220 °C; sniff port humidity control, 100 mL/min moist He.

For gas-phase separations employing a chiral stationary phase, similar injection and chromatographic parameters were employed.

GC/MS Analysis of Distillates. Samples obtained by fractional distillation of natural davana oil (as received) at reduced pressure (1 mmHg) were analyzed using an Agilent (HP) 6890 GC equipped with an Agilent 5973 mass selective detector. The column was a 30 m DB-1 (J&W Scientific Co.) with a 250 μm diameter and a 0.25 μm film thickness. Injection temperature was 250 °C. The distillate (1 μL) was injected using a 10:1 split ratio in constant flow mode (1 mL/min). Initial oven temperature was set to 50 °C, after which the temperature was ramped to 240 °C at 4 °C/min. The mass spectrometer detector scan was from 33 to 300 mass units. Only one injection was made per sample.

Preparative Supercritical Fluid Chromatography/Ultraviolet Spectroscopy (SFC/UV). The crude davana oil sample was preliminarily fractionated prior to semipreparative chromatography to maximize the high-efficiency separation. Initially, 5 g of oil was mixed with both 20 mL of hexane and 20 mL of 70:30 ethanol/H_2O. After 5 min of extraction, the ethanol/H_2O fraction was separated from the hexane, which was then extracted a second time with fresh ethanol/H_2O. This procedure was repeated a total of three times. All three ethanol/H_2O fractions were combined. Then most of the ethanol and a small amount of water were removed from the mixture via rotary evaporation. Approximately 1 g of ethyl acetate was next added to the resulting aqueous mixture followed by subsequent addition of CH_2Cl_2 (20 mL). At this point davanone and other residual compounds were extracted into the CH_2Cl_2. This procedure was repeated a second time, after which the combined extract was reduced in volume again via rotary evaporation. This concentrated methylene chloride sample was then further cleaned up by passing it through a silica gel column (150 × 10 mm) using 150 mL of hexane. The hexane wash, which was believed to contain mostly davanone at this point, was reduced in volume. The concentrated hexane solution was then subjected to semipreparative SFC for further purification of davanone.

Various analytical-scale, silica-based stationary phases were tested initially to determine which provided the better separation. Each column was 4.6 mm × 25 cm with a particle size equal to 5 μm. The results of that study suggested that the 2-ethylpyridine column provided the best separation with the least amount of methanol modifier in the mobile phase. The semipreparative separation was consequently next developed on a semipreparative 2-ethylpyridine column (250 × 10 mm, 5 μm d_p, Princeton Chromatography, Inc.). Detection was obtained using a UV detector at 205 and 214 nm. The following chromatographic conditions were employed for separation of the davanone: pressure, 120 atm; oven temperature, 45 °C; flow, 4 mL/min for the first 10 min and then 5 mL/min for the next 7 min; injection volume, 40 μL of davanna oil liquid−liquid extract; modifier, methanol. The modifier schedule was as follows: initial, 99/1% CO_2/MeOH; hold for 8 min; ramp to 96/4% CO_2/MeOH at rate of 1%/min; ramp to 20% at rate of 20%/min; hold for 3.5 min; ramp down to 99/1% at 50%/min; and hold for 1.5 min.

Figure 2 shows the semipreparative SFC/UV trace for separation of the extract. Three fractions were collected. Fraction 2 contained mostly davanone. The initial and final collection points for each fraction are noted in the figure. Fractions 1 and 3 did not contain any davanone. After completion of preparative SFC, several milligrams of each collected fraction was diluted to 10 mL with CH_2Cl_2 and analyzed via GC/MS. GC separations were performed on a DB-5 MS capillary column (15 m × 0.25 mm i.d., 0.25 μm d_f). All GC runs were carried out using the following temperature program: initial temperature, 80 °C; hold for 2 min; ramp to 140 °C at 10 °C/min; ramp to 290 °C at 4 °C/min; hold at 290 °C for 10 min. The split/splitless injection volume was 1 μL with a split ratio of 1:10. Injection and detector temperatures were set to 290 °C.

RESULTS AND DISCUSSION

Distillation of Natural Davana Oil. Our first attempt concerning natural davana oil separation and characterization dealt with distillation at both atmospheric and reduced pressures. Both ambient and reduced pressure distillations were attempted. Both led to color change and fuming, clearly indicative of decomposition. Fraction 6, on analysis by GC/MS, gave the highest percentage of davanone (all fractions, regardless of decomposition indications, were analyzed by GC/MS). This fraction appeared to contain the least number of components, with many of the early-eluting peaks seen in earlier fractions now absent from this chromatogram. Using mass spectral analysis, numerous peaks from fraction 6 were identified in addition to davanone. The davanone response was strongest and split into at least four peaks, which suggested the presence of multiple isomeric forms. The remainder of this fraction was populated with numerous other compounds but at significantly lower abundance. From these experiments, it was concluded that distillation was not an acceptable method for isolation of a pure fraction of davanone from natural davana oil. Thus, supercritical fluid extraction became an attractive alternative methodology (vide infra).

Headspace SPME/GC/MS of Natural Davana Oil. Analysis of the headspace above davana oil using automated SPME/GC/

MS revealed the presence of a wide array of volatile components that obviously contribute to some degree to the aroma associated with davana oil. The presence of major headspace volatiles is consistent with the reported composition of davana oil (*1*). **Figure 3** presents the total ion chromatogram, which gives an overview of the major components detected in the headspace. In addition to davanone, bicyclogermacrene, linalool, and several aliphatic esters were major components identified. Information concerning the structure of some of the minor components detected in the headspace above the davana oil is given in **Table 1**. Two criteria were employed to assign the identification as tentative: (a) library search quality >80% and (2) inability to interpret the mass spectrum so as to yield an acceptable level of confidence in assigning a structure. The presence of acetaldehyde, furan, and furanone derivatives could possibly indicate some degree of thermal degradation during the preparation of the davana oil. Low levels of acetaldehyde are suspected to contribute to "off-notes" in essential oils (*16*, *17*). Davanone-related components eluted rather late in the experiment: nordavanone (peak 48) at 13.17 min, davanone isomers (peaks 56 and 62) at 15.01 and 16.72 min, davana ethers (peaks 58 and 60) at 15.69 and 16.10 min, and *cis*-davanone (peak 64) at 17.29 min. Only *cis*-davanone of these six peaks gave a strong MS response. The major components listed in **Table 1** are consistent with those previously reported for davana oil (*22*). Although davanone could be chromatographically identified, isolation of significant quantities for future study was not realized.

GC/MS/O of Natural Davana Oil. The major components identified from the total ion chromatogram (TIC) of commercial davana oil dissolved in methylene chloride are given in **Figure 4**. The sensory notes detected via GC of the CH_2Cl_2 solution of davana oil are also illustrated in **Figure 4**. The bold numbers describe the intensity of the sensory note on a scale of 0−100. A greater numerical value signifies a greater intensity of the sensory note. Sensory notes were determined by one individual who had had 5 years of experience in GC/O technologies. Informal aroma dilution analyses were performed until the intensity values were <100. The most important odor-active compounds were ascertained by noting the intensity values obtained via GC/O. A number of important observations can be made from the olfactometry evaluation of the crude natural davana oil: (1) major components do not necessarily correlate with major sensory responses; (2) powerful sensory responses are observed for minor/trace constituents; (3) davanone with a value of 40 is not the major sensory component, yet it affords the greatest MS response; (4) eight other components had intensities greater than that of davanone; and (5) a number of powerful sensory components are essentially below TIC detection limits and, hence, remain unidentified at this time. In addition, all of the sensory attributes identified in the GC/O analyses were positive in nature and could be described as fruity, spicy, musty, green, sweet, and pleasant. These olfactometry results are in agreement with the literature in that davanone has been reported to have little to no aroma. On the other hand, minor components are thought to contribute significantly to the aroma of davana oil (*1*, *6*, *23*).

Chiral GC/MS Analysis of Davanone Fraction Isolated by Preparative SFC. Four chiral stationary phases were employed to evaluate the chiral purity of the davanone fraction isolated by means of preparative supercritical fluid chromatography. Selected Restek chiral columns, which incorporate various combinations of alkylated β-cyclodextrins into a cyanopropyl-dimethylpolysiloxane liquid stationary phase, were studied as follows: (1) RT-β-dexse, (2) RT-β-dexsp, (3) RT-β-dexsm, and (4) RT-β-dexcst. With each chiral column, a similar separation of pure davanone was obtained. A racemic mixture of octalactone, which exhibits one chiral center, was employed in the mixture to document the chiral performance of each column because, surprisingly, only one GC/MS peak

Table 1. Components Identified in Natural Davana Oil Headspace Using SPME/GC/MS

peak	compound	retention time (min)	area %
1	acetaldehyde	1.80	<0.50
2	octane	2.19	<0.50
3	acetone	2.36	2.02
4	nonene	3.41	<0.50
5	ethanol	3.46	<0.50
6	3-methyl-3-buten-2-one[a]	4.12	<0.50
7	α-pinene	4.38	<0.50
8	α-thujene	4.48	<0.50
9	2-ethyl-5-methylfuran	4.60	<0.50
10	ethyl-2-methylbutyrate	4.90	2.24
11	ethyl-3-methylbutyrate	5.14	1.27
12	5-ethyl-2-methyl-2-vinyltetrahydrofuran	5.19	1.38
13	β-pinene	5.60	<0.50
14	sabinene	5.83	<0.50
15	5-ethyl-2-methyl-2-vinyltetrahydrofuran, isomer	6.03	4.61
16	propyl-2-methylbutyrate	6.15	3.85
17	propyl-3-methylbutyrate	6.39	3.01
18	α-phellandrene	6.49	<0.50
19	α-terpipene	6.70	1.57
20	2-methylpropyl-3-methylbutyrate	6.98	<0.50
21	1,8-cineole	7.15	<0.50
22	5-isopropyl-2-(2-methyl-2-vinyl)tetrahydrofuran	7.20	<0.50
23	5-ethyl-2-methyl-2-vinyltetrahydrofuran, isomer	7.46	2.20
24	γ-terpinene	7.68	1.81
25	*trans*-β-ocimene	7.78	<0.50
26	5-ethyl-2-methyl-2-vinyltetrahydrofuran, isomer	7.92	2.65
27	*p*-cymene	8.06	3.28
28	2-methylbutyl-2-methylbutyrate	8.20	4.58
29	pentyl-3-methylbutyrate	8.42	2.17
30	6-methyl-5-hepten-2-one	9.06	<0.50
31	3-methyl-3-butenyl-3-methylbutyrate	9.46	<0.50
32	unknown	9.80	<0.50
33	unknown	10.10	<0.50
34	acetic acid[a]	10.60	1.13
35	sabinene hydrate	10.76	0.64
36	unknown	10.83	<0.50
37	α-copaene	11.08	<0.50
38	unknown	11.14	<0.50
39	α-gurjunene	11.56	<0.50
40	benzaldehyde	11.63	1.03
41	linalool	11.77	4.66
42	β-elemene	12.32	1.23
43	β-caryophyllene	12.43	0.62
44	terpinen-4-ol	12.51	4.07
45	aromadendrene	12.55	<0.50
46	5,5-dimethyl-2(5*H*)-furanone[b]	12.69	1.61
47	alloaromadendrene	13.02	1.95
48	nordavanone	13.17	0.91
49	5-ethenyldihydro-5-methyl-2(3*H*)-furanone	13.44	1.21
50	ledene	13.60	1.59
51	germacrene	13.78	1.81
52	β-selinene	13.90	4.45
53	bicyclogermacrene	14.07	17.60
54	geranyl acetate	14.27	3.10
55	α-amorphene	14.33	<0.50
56	davanone isomer	15.01	<0.50
57	methylhydrocinnamate	15.34	<0.50
58	davana ether isomer	15.69	<0.50
59	ethylhydrocinnamate	15.75	<0.50
60	davana ether isomer	16.10	<0.50
61	methylcinnamate	16.51	<0.50
62	davanone isomer	16.72	<0.50
63	ethylcinnamate	16.86	1.31
64	*cis*-davanone	17.29	13.63

[a] Coelution. [b] Tentative identification.

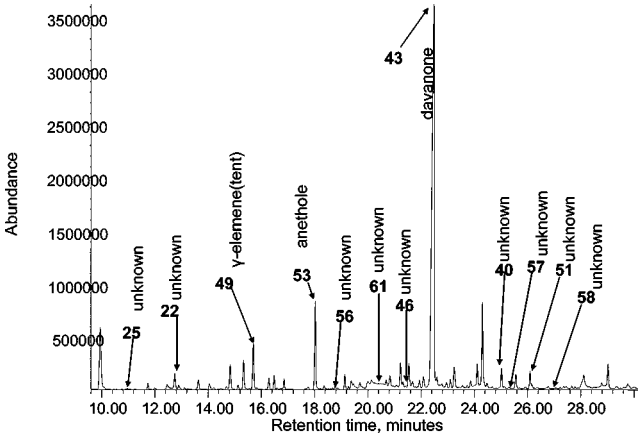

Figure 4. Total ion chromatogram for natural davana oil with aroma responses shown in bold numbers.

was assignable to purified davanone which had been produced by preparative SFC (**Figure 5A**). A single peak to the right of the davanone peak was observed, which can be assigned to ethyl cinnamate as would be expected because this compound does not have a chiral center. Injections of successive dilutions of the purified davanone sample continued to reveal the presence of a single component, which suggested the absence of additional optical isomers. Thus, initial processing of natural davana oil through a supercritical fluid treatment seems to have eliminated some of the davanone isomers and other components that were prominently displayed when natural davana oil was subjected to chiral GC/MS (vide infra). The chiral retention time for the davanone isolated by SFC, 49.75 min, was exactly the same chiral retention time as observed for the major davanone peak from davana oil, 49.76 min. Contributions from the other possible related components were not observed in the SFC isolated davanone. The failure to observe two chiral GC/MS peaks for the fractionated davanone suggested that the davanone may be optically pure. Small responses close to the SFC davanone fraction possessed some of the features of the mass spectrum of davanone, but the spectral response was not intense enough to make a definitive identification. Polarimetric measurement of the davanone sample isolated by preparative SFC/UV at 27 °C and 598 nm (0.1484 g/2 mL) gave a specific optical rotation, [α], equal to +63.45, a value considerably higher than the rotation found for natural davana oil.

Chiral GC/MS Analysis of Natural Davana Oil. For comparison, chiral GC/MS analysis of natural davana oil (**Figure 5B**) prior to preparative SFC with the same stationary phases as used with the purified davanone revealed the presence of at least three sets of stereoisomers (**Figure 6**). Set C corresponded to davanone with retention times at 47.5, 48.0, 48.4, and 49.8 min (i.e., peaks c1, c2, c3, and c4). The distribution of these isomers within the set was 0.88, 2.50, 0.85, and 95.76%, respectively. Davanone exhibits three chiral carbon centers at carbons 6, 7, and 10 (structure A). The final eluting component no doubt is the 6*S*,7*S*,10*R* isomer that has previously been reported (*3*). Therefore, theoretically there should be four diastereomeric pairs of enantiomers associated with the davanone molecule. All four peaks that were observed exhibited the davanone mass spectrum and are no doubt attributable to these four diastereomers. Set A is assigned to the four diastereomers of davana ether (structure B) (*23*), although nordavanone (*24*) and davanafuran (*25*) cannot be ruled completely out. The mass spectra hit qualities for davana ether, however, were all >95%. Because the mass spectrum for the davanafuran would not be the same as that for the davana ether (which also has 2 fewer mass units), the original assignment is the correctly identified compound. Retention times equal to 43.12, 44.88, 45.07, and 46.36 min (i.e., peaks a1, a2, a3, and a4) are observed. The distribution for these isomers within the group was 16.09, 10.49, 45.05, and 28.38%, respectively. The presence of four optical isomers for davanone and davana ether is consistent with previously reported literature (*8*, *9*). Set B was assignable to 2-(2-methyl-2-vinyltetrahydrofur-5-yl)-2,6,6-trimethyl-2,6-dihydropyr-3-one (structure C) with retention times at 45.21 and 45.74 min (i.e., peaks b1 and b2). This oxidized,

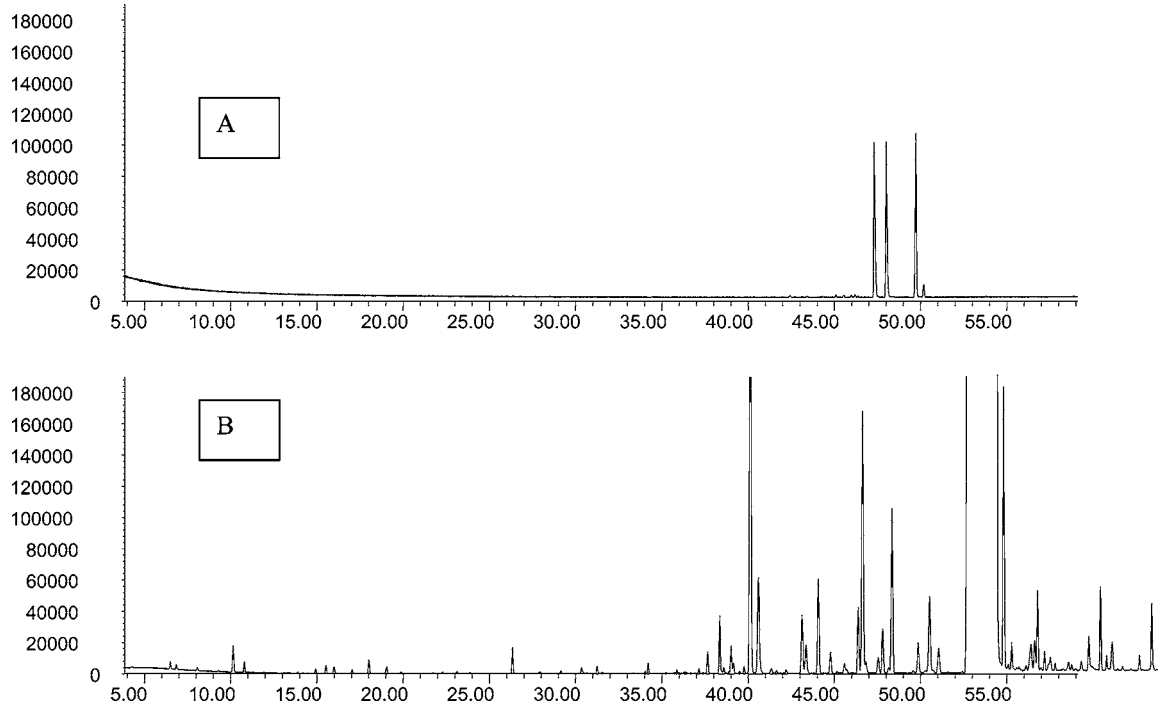

Figure 5. Chiral total ion GC/MS chromatogram of natural davana oil and davanone isolated via preparative SFC, β-dexcst stationary phase (30 × 0.25 mm, i.d. × 0.25 μm film thickness).

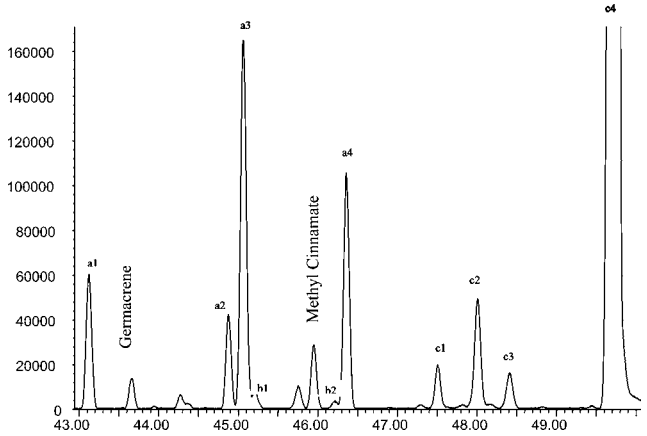

Figure 6. Expanded chiral total ion GC/MS of natural davana oil.

Table 2. ^{13}C Chemical Shift Assignments for Davanone Fraction Isolated by Preparative SFC

	^{13}C chemical shift	
carbon	current study	ref *3*
1	17.8	17.8
2	134.9	134.9
3	116.0	116.0
4	42.4	42.4
5	208.6	208.6
6	51.32	51.0
7	80.8	80.8
8	29.7	29.7
9	37.3	37.3
10	83.03	82.8
11	144.0	144.0
12	111.1	111.1
13	25.6	25.6
14	13.0	13.0
15	26.4	26.4

cyclized derivative of davanone has been previously reported (*26*). The distribution of these isomers within the group was 21.90 and 78.10%, respectively. The two peaks arising from set B were weak. This may account for why four peaks were not observed with the unsymmetrical molecule that has two chiral centers. Polarimetric measurement at 27 °C and 589 nm of the natural davana oil (0.1559 g/2 mL) revealed an enantiomeric excess of one (or more) of the components. The specific optical rotation, [α], was equal to +51.37, which was significantly lower than the optical rotation that was observed for the davanone fraction isolated via SFC.

NMR Analysis of Davanone Fraction Isolated by Preparative SFC. Using the numbering scheme depicted in **Figure 1A**, ^{13}C chemical shift analysis assignments were made for the davanone fraction isolated via SFC (**Table 2**). Excellent agreement between the chemical shift data set measured on purified davanone and that reported much earlier was realized. Thus, the isolated davanone in this work is consistent with the *cis*-davanone isomer (*3*). It should be noted, however, that the isolated *cis*-davanone was not indefinitely stable in the deuterated chloroform NMR solvent because it appeared to degrade significantly after a period of 7 days. This finding is consistent with previously reported studies which claim that autoxidation of davanone when left open to the air leads to polymeric materials that are not easily chromatographed (*23*). Preliminary proton NMR studies of this phenomenon in this work also indicated the presence of polymerization-type reactions.

In summary, the components of natural davana oil have been thoroughly characterized by the application of numerous analytical techniques. The major constituent, davanone, has been separated from the bulk matrix by carbon dioxide-based packed column semipreparative supercritical fluid chromatography. The purified davanone exhibits high optical rotation, which no doubt accounts for our observation of a single chiral chromatographic peak. A different observation was made when chiral GC was performed on the natural davana oil sample. Peaks for all four anticipated diastereomers were observed along with isomers of davana ether. Nuclear magnetic resonance spectroscopic measurements of purified davanone are consistent with the cis structure. Gas chromatography coupled with olefactometry indicated the presence of a number of minor components in natural davana oil that have higher sensory activity than any of the davanone isomers.

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LITERATURE CITED

(1) Mallavarapu, G. P.; Kulkarni, R. N.; Baskaran, K.; Rao, L.; Ramesh, S. Influence of plant growth stage on the essential oil content and composition in davana (*Artemisia pallens* Wall.). *J. Agric. Food Chem.* **1999**, *47*, 254−258.

(2) Catalan, C. A. N.; Cuena, M. D.; Verghese, J.; Joy, M. Y.; Guturrez, A. B.; Herz, W. Sesquiterpene ketones related to davanone from *Artemisia pallens*. *Phytochemistry* **1990**, *29*, 2702−2703.

(3) Thomas, A. F.; Thommen, W.; Willhalm, B.; Hagaman, E. W.; Wenkert, E. Terpenoids derived from linalyl oxide. Part I. The stereochemistry of the davanones. *Helv. Chim. Acta* **1974**, *57*, 2055−2061.

(4) Guenther, E. *The Essential Oils, Vol. 5, Oil of Davana*; Litton Educational Publishing: New York, 1952; p 451.

(5) Lamparskey, D.; Klimes, I. Natural flavoring materials—davana oil: some analytical results with respect to biogenetically plausible structural features. In *Topics in Flavor Research*; Berger, R. G., Nitz, S., Schreir, P., Eds.; Proceedings of an International Conference, April 1−2, 1985, Freising-Weihenstephan, Germany; H. Eichorn Publishing: Freising, Germany, 1985; pp 281−304.

(6) Thomas, A. F.; Pitton, G. The isolation, structure, and synthesis of davana ether, an odoriferous compound of the oil of *Artemisia pallens* Wall. *Helv. Chim. Acta* **1971**, *54*, 1890−1891.

(7) Ramezani, M.; Behravan, J.; Yazdinezhad, A. Chemical composition and antimicrobial activity of the volatile oil of *Artemisia khorassanica* from Iran. *Pharm. Biol.* **2004**, *42*, 599−602.

(8) Sadeghpour, O.; Asghari, G.; Ardekani, M. R. S. Composition of essential oil of *Artemisia persica* Boiss, from Iran. *Iranian J. Pharm. Res.* **2004**, *3*, 65−67.

(9) Certificate of Analysis Sheet, Davana Essential Oil, Product 1852, *Artemisia pallens*; Liberty Natural Products; available at http://www. libertynatural.com.

(10) Bartlett, P. A.; Holmes, C. P. A highly stereospecific synthesis of davanone. *Tetrahedron Lett.* **1983**, *24*, 1365−1368.

(11) Birch, A. J.; Corrie, J. E. T.; Rao, G. S. R. A non-stereospecific synthesis of (±)-davanone. *Aust. J. Chem.* **1970**, *23*, 1811−1817.

(12) Ashraf-Khorassani, M.; Nazem, N.; Taylor, L. T.; Coleman, III, W. M. Isolation of tetra-acyl sucrose esters from Turkish tobacco using supercritical fluid CO_2 and comparison with conventional solvent extraction. *J. Agric. Food Chem.* **2005**, *53*, 1866−1872.

(13) McHugh, M. A.; Krukonis, V. J. *Supercritical Fluid Extraction*, 2nd ed.; Elsevier: Amsterdam, The Netherlands, 1994.

(14) Westwood, S. S. Ed. *Supercritical Fluid Extraction and its Use in Chromatographic Sample Preparation*; Blackie Academic and Professional: London, U.K., 1993.

(15) Coleman III, W. M.; Dube, M. F. Headspace solid-phase microextraction analysis of artificial flavors. *J. Sci. Food Agric.* **2005**, *85*, 2645−2654.

(16) Coleman III, W. M.; Lawrence, B. M.; Cole, S. K. Semi-quantitative determination of off-notes in mint oils by solid-phase microextraction. *J. Chromatogr. Sci.* **2002**, *40*, 133−139.
(17) Coleman III, W. M.; Lawrence, B. M.; Craven, S. H. The use of a non-equilibrated solid phase microextraction method to quantitatively determine the off-notes in mint and other essential oils. *J. Sci. Food Agric.* **2004**, *84*, 1223−1228.
(18) LeLand, J. V.; Schieberle, P.; Buettner, A.; Acree, T. E. *Gas Chromatography−Olfactometry State of the Art*; ACS Symposium Series 782; American Chemical Society: Washington, DC, 2001.
(19) Chisholm, M. G.; Wilson, M. A.; Gaskey, G. M.; Jell, J. A.; Cass Jr., D. M. The identification of aroma compounds in key lime oil using solid-phase microextraction and gas chromatography−olfactometry. In *Gas Chromatography−Olfactometry State of the Art*; ACS Symposium Series 782; LeLand, J. V., Schieberle, P., Buettner, A., Acree, T. E., Eds.; American Chemical Society: Washington, DC, 2001.
(20) Jelen, H. H.; Majcher, M.; Wasowicz, E. Analysis of microbial volatile metabolites responsible for musty-earthy odors by headspace solid-phase microextraction gas chromatography/mass spectrometry. In *Handbook of Flavor Characterization, Sensory Analysis, Chemistry and Physiology*; Deobler, K. D., Delwiche, J., Eds.; Dekker: New York, 2004.
(21) Pandya, R. N.; Peppard, T. L. Review of analytical methodologies for volatile nitrogen heterocycles in food. In *Heteroatomic Aroma Compound*; ACS Symposium Series 826; Reineccius, G. C., Reineccius, T. A., Eds.; American Chemical Society: Washington, DC, 2002.
(22) Burdock, G. A. Davana and davana oil. *Fenaroli's Handbook of Flavor Ingredients*, 4th ed.; CRC Press: New York, 2002; p 379.
(23) Benjamin, B. D.; Sipahimalani, A. T.; Heble, M. R. Tissue cultures of *Artemisia pallens*: organogenesis, terpenoid production. *Plant Cell, Tissue Organ Culture* **1990**, *21*, 159−164.
(24) Thomas, A. F.; Dubini, R. Terpenoids derived from linalyl oxide, Part 4. The oxidation of davanone. Isolation and synthesis of the davana ethers, sesquiterpenes of *Artemisia pallens*. *Helv. Chim. Acta* **1974**, *57*, 2076−2081.
(25) Thomas, A. F.; Ozainne, M. Terpenoids derived from linalyl oxide. Part 2. The isolation and synthesis of nordavanone, a C_{11}-terpenoid from *Artemisia pallens*. *Helv. Chim. Acta* **1974**, *57*, 2062−2065.
(26) Thomas, A. F.; Dubini, R. Terpenoids derived from linalyl oxide. Part 3. The isolation, structure, absolute configuration and synthesis of the davanafurans, nor-sesquiterpenes isolated from *Artemisia pallens*. *Helv. Chim. Acta* **1974**, *57*, 2066−2075.
(27) Thomas, A. F. Terpenoids derived from linalyl oxide. Part 5. 2-(2-methyl-2-vinyltetrahydrofur-5-yl)-2,6,6-trimethyl-2,6-dihydropyr-3-one, a new sesquiterpenoid isolated from *Artemisia pallens*. *Helv. Chim. Acta* **1974**, *57*, 2081−2083.

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