The Composition of *Dalea formosa* Oil Determined by Steam Distillation and Solid-Phase Microextraction

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Abstract

Dalea formosa Torr. (feather dalea, featherplume) was collected from the Jornada Experimental Range in south central New Mexico. Current year's growth was collected from 10 plants, all found within an approximate 50 m radius of the GPS coordinates N32°40.645' and W106°33.601' during July 2001. Composite samples of the plants were steam distilled in triplicate, and the composite oil was analyzed using both GC-FID and GC/MS. The volatile composition of plants collected from the same site was also examined using solid-phase microextraction (SPME) with a 100 μm polydimethylsiloxane fiber. Mass spectra and retention indices were used to identify 58 previously described compounds. The retention index and EI mass spectra are provided for one unknown. The most abundant constituents of the oil were α-pinene (31.7%), camphene (8.4%) and limonene (8.1%). In contrast, α-pinene (33.6%), β-pinene (13.2%) and camphene (11.1%) were the most abundant constituents of the SPME samples. This difference in composition may be due to either sampling technique or harvesting time.

Key Word Index

Dalea formosa, Fabacae, feather dalea, essential oil composition, α-pinene.

Introduction

Dalea formosa Torr., commonly known as feather dalea, is a woody shrub of the family Fabaceae found on rocky hillsides from southeastern Colorado to northern Mexico, and from Arizona east to Oklahoma and Texas. The plant has been described as good native browse for deer (1) and antelope (2). The *Dalea* genus is large, consisting of at least 250 species in the Americas (3), yet only a few of these have been examined chemically. Of those species that have been studied, phenolic compounds have been emphasized (3-6), with little attention given to the volatile fraction. We found no published essential oil compositions for any member of the *Dalea* genus.

Our group is working to describe the volatile compounds of Chihuahuan Desert plants (7-10) and explore interactions between plant volatiles and large herbivores (11-13). In the oil compositions we have published to date, oils have been isolated using steam distillations of above-the-ground tissues or ethanol extraction of leaf-surface volatiles. Steam distillation is a tedious approach that is difficult to apply to large numbers of samples. This has restricted our ability, with available resources, to examine plant-to-plant and seasonal variation of oil production. In *Flourensia cernua* D.C., ethanol extraction of leaf surfaces provided oil profiles similar to those obtained by steam distillation (8). However, with some plant species, ethanol extracts contain many semi-volatile compounds that are difficult to remove from GC columns and injectors (unpublished data). Therefore, a second objective of this study was to explore the ability of solid phase microextraction (SPME) to detect a broad range of plant volatiles.

Experimental

Plant material was collected from the USDA-ARS Jornada Experimental Range in southern New Mexico, at an altitude of 1,728 m above sea level. Ten plants were randomly selected from within an approximate 50 m radius of the GPS coordinates N32°40.645' and W106°33.601'. Coordinates were determined using a Garmin GPS 12 personal navigator. Samples consisted of 20 15 cm leaders of current year's growth from each of 10 plants. These samples were collected on July 27, 2001, immediately placed on dry ice, and stored at -20°C until steam distillations and dry matter analyses were performed. A voucher specimen identified as *Dalea formosa* Torr. was placed in the Department of Animal and Range Science Herbarium located at New Mexico State University in Las Cruces, NM.

Leaf and small stem tissues (approximately 1 mm in diameter) from the 10 plants were combined and ground to a coarse powder in liquid nitrogen. Three separate batches weighing between 15 and 20 g were steam distilled for 6 h as previously described (10), using a 500 mL flask and 250 mL water. The oil retrieved from each distillation was dissolved in 100% ethanol for GC/MS analysis, and injected as pure oil for GC-FID analysis.

An unplanned loss of plant material during SPME optimization resulted in the need for a second collection of plant

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material. Plant tissue was collected from the same site, in the same manner as described above. However, it is critical to note that the second collection occurred on August 31, 2001. For this reason, quantitative comparisons of the results obtained from the two methods are not possible. Nonetheless, SPME results are included to demonstrate the utility of solid-phase microextraction for detecting a range of plant volatiles comparable to those detected in the steam distilled oils.

SPME was performed in triplicate by equilibrating 0.5 g of plant tissue (ground with liquid nitrogen as above) in 4 mL autosampler vials with PTFE/silicon septa for 2 h at 50°C. A 100 μm polydimethylsiloxane (PDMS) SPME fiber purchased from Supelco (Bellefonte, PA) was placed in a manual fiber holder (also from Supelco) and exposed to the headspace of the sample for 15 min at a depth of 1 cm. The fiber was injected into the GC/MS and desorbed for 3 min. Blank injections followed each sample to verify the absence of residual compounds on the SPME fiber. The fiber was then re-exposed to the sample as before, injected into a GC-FID, and desorbed for 3 min.

GC/MS analysis was performed using a Varian model 3400 GC with a DB-5 column (30 m x 0.25 mm fused silica capillary column, film thickness 0.25 μm) coupled to a Finnigan ion trap mass spectrometer (EI, 70 eV). Helium at approximately 1 mL/min was used as a carrier gas, and injector and transfer line temperatures were set at 220°C and 260°C, respectively. The initial column temperature was 60°C, and a linear temperature increase of 3°C/min was programmed into each 65 min run. When injecting oils, a series of large (500 ng) to small (50 ng) injections were used to validate retention times for both low- and high-concentration components. Compounds were identified by comparing mass spectra and retention indices with literature data (14,15) and with our own MS library, which was developed using authentic standards.

To validate peak area percentages revealed on the total ion chromatogram, the oil was also analyzed using a Shimadzu GC8APF equipped with a flame ionization detector and fitted for use with capillary columns. A split/splitless injector was used, and the column type, temperature gradients, and helium flow rate were identical to those used for the GC/MS analysis. The injector temperature was 250°C. Dry matter percent was determined for the tissues that were steam distilled by drying triplicate, 2 g samples of ground tissue at 100°C for 24 h.

Results and Discussion

Dry matter accounted for 82.5% of the tissue fresh weight. The steam distillate comprised 7.2 mg/g of dry matter. Table I shows the identities, retention indices (RI), and the percent composition (by FID) of all the oil components that were identified in either steam distilled oil or SPME injections. Positive identification required both an RI within five units of reported values (9,14,15), and an MS library fit score greater than 950. In some cases, peaks that appeared clearly in the mass spectrometer were not detected by FID. Fifty-eight compounds were positively identified, accounting for 87.1% of the composition of the steam-distilled oil. Unknowns (8.2%) and unresolved peaks (4.8%) accounted for the remainder of the total detected peak area. There were numerous unknowns, with 98 total peaks detected by GC/MS. However, most of these were too small to provide clear mass spectra. Only one unknown, with a RI of 1333, provided a high enough signal-to-noise ratio by to confidently report its spectrum. This peak made up 0.09% of the oil, and 0.01% of the SPME chromatograms. The EI spectrum for this unknown is 41(25), 43(100), 67(13), 77(15), 79(30), 91(27), 93(79), 67(16), 108(31), 121(61), 136 (13). Since soft ionization was not available, no molecular ion was designated.

The percent compositions of individual compounds detected following SPME injections are in many cases quite different from the percent compositions observed in the oil. For example, with SPME, 92.21% of the mean total peak area was accounted for by known compounds, 5.2% were unknowns and 2.8% were unresolved peaks. One obvious difference was the percentage of β-pinene in the SPME chromatograms (13.2% vs. 5.8% of the oil). Other compounds with concentrations notably higher in SPME chromatograms include tricyclene, camphene, o-cymene, and α-copaene. Since the SPME samples were from a different batch of plant tissue, it cannot be determined whether these differences are due to differences in the tissue or in the sample preparation technique. However, it is important to add that even though not all oil compounds shown (Table I) were detected with SPME by FID, they were all detected in the SPME by GC/MS. This observation combined with literature reports (16-18) assures us that SPME can fill a valuable role in sampling plant volatiles. The ease with which SPME analysis can be automated makes it an ideal tool for more complex studies examining spatial and temporal variation in rangeland volatiles.

α-Pinene, the most abundant compound in both oil and SPME chromatograms, represented approximately one third of the oil. α-Pinene has been demonstrated to deter feeding by sheep when applied to alfalfa pellets (13). Camphene, a potent antioxidant, β-pinene, and limonene are also found in high quantities. Cytotoxicity of α- and β-pinene and limonene towards human tumor cell lines was recently demonstrated (19). It is worth noting that these three compounds constitute 45.7% of the *Dalea formosa* oil.

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Table I. Percentage composition of the oil and headspace (SPME)of *Dalea formosa*

RI	Compound	Oil (SD)	Headspace (SPME)	RI	Compound	Oil (SD)	Headspace (SPME)
799	hexanal	0.0 (0.0)	ND (NA)	1190	α-terpineol	0.6 (0.1)	0.1 (0.0)
869	hexanol	ND (NA)	0.1 (0.0)	1221	α-fenchyl acetate	0.4 (0.0)	0.1 (NA)
930	tricyclene	2.0 (1.4)	3.7 (0.3)	1238	3-methyl-3-hexyn-1-yl butyric acid	0.1 (0.0)	0.0 (NA)
932	α-thujene	0.5 (0.4)	0.4 (0.0)	1244	carvone	ND (NA)	0.0 (NA)
942	α-pinene	31.7 (1.2)	33.6 (1.9)	1248	carvotanacetone	ND (NA)	ND (NA)
957	camphene	8.4 (2.8)	11.1 (0.7)	1273	perillaldehyde	ND (NA)	ND (NA)
982	β-pinene	5.8 (1.0)	13.2 (0.5)	1286	bornyl acetate	4.5 (0.6)	1.0 (0.0)
987	6-methyl-5-hepten-2-one	ND (NA)	ND (NA)	1312	*cis*-pinocarvyl acetate	0.1 (0.1)	0.0 (0.0)
993	myrcene	2.4 (0.4)	2.2 (0.1)	1350	α-terpinyl acetate	0.3 (0.0)	0.2 (NA)
1007	α-phellandrene	1.9 (0.7)	1.4 (0.0)	1377	α-copaene	0.1 (0.1)	1.1 (0.0)
1014	δ-3-carene	ND (NA)	ND (NA)	1392	β-elemene	0.1 (0.0)	0.1 (NA)
1020	α-terpinene	0.3 (0.1)	0.1 (0.0)	1419	β-caryophyllene	2.6 (0.8)	8.0 (0.1)
1028	p-cymene	0.9 (0.1)	2.2 (0.1)	1454	α-humulene	0.2 (0.1)	0.5 (0.0)
1033	limonene	8.2 (0.6)	7.4 (0.3)	1476	γ-muurolene	0.2 (0.1)	0.5 (0.0)
1041	(Z)-β-ocimene	0.5 (0.2)	0.3 (0.0)	1480	γ-curcumene	0.2 (0.0)	0.6 (0.1)
1044	phenylacetaldehyde	ND (NA)	ND (NA)	1483	germacrene D	0.1 (0.4)	0.1 (NA)
1052	(E)-β-ocimene	0.7 (0.2)	ND (NA)	1485	β-selinene	0.8 (0.5)	0.9 (0.0)
1062	γ-terpinene	4.1 (1.2)	1.1 (0.1)	1494	α-selinene	1.0 (0.1)	1.1 (0.1)
1090	terpinolene	1.1 (0.2)	ND (NA)	1508	(E-E)-α-farnesene	0.1 (0.0)	0.1 (0.0)
1099	linalool	0.2 (0.0)	0.1 (0.0)	1512	β-curcumene	0.1 (0.0)	0.4 (0.0)
1123	*cis*-p-menth-2-en-1-ol	0.1 (0.0)	0.0 (0.0)	1513	γ-cadinene	0.1 (0.0)	0.0 (0.0)
1127	α-campholenal	ND (NA)	0.0 (0.0)	1517	7-epi-α-selinene	0.1 (0.7)	ND (NA)
1141	*trans*-p-menth-2-en-1-ol	0.1 (0.0)	0.0 (0.0)	1525	zonarene	0.2 (0.1)	ND (NA)
1146	camphor	0.0 (NA)	0.0 (0.0)	1532	cadina-1(2),4-diene	0.1 (0.1)	0.2 (0.0)
1150	camphene hydrate	0.1 (0.0)	ND (NA)	1582	caryophyllene oxide	0.2 (NA)	ND (NA)
1164	pinocarvone	ND (NA)	ND (NA)	1631	γ-eudesmol	0.5 (0.1)	0.0 (NA)
1174	isopinocamphone	ND (NA)	ND (NA)	1649	β-eudesmol	3.2 (1.3)	0.0 (NA)
1178	terpinen-4-ol	0.3 (0.1)	0.1 (NA)	1653	α-eudesmol	2.2 (NA)	0.1 (0.0)
1187	p-cymen-8-ol	0.1 (0.0)	0.0 (NA)	1666	bulnesol	0.0 (NA)	ND (NA)

1 ND identifies compounds which were detected by GC/MS but could not be detected by FID; NA indicates that a standard deviation could not be calculated, either because the compound was not detected or because it was only detected in one sample; 0.0 means the compound was detected, but comprised less than 0.05% of the averaged peak area percent

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