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Volatile Composition of *Gutierrezia sarothrae* (Broom Snakeweed) as Determined by Steam Distillation and Solid Phase Microextraction

Mary E. Lucero,* Ed L. Fredrickson and Rick E. Estell

USDA ARS Jornada Experimental Range, Box 30003, MSC 3JER, Las Cruces, New Mexico 88003; e-mail: malucero@nmsu.edu

Andrine A. Morrison and David B. Richman

Department of Entomology, Plant Pathology, and Weed Science, New Mexico State University, Box 30003, MSC 3BE, Las Cruces, New Mexico 88003

Abstract

Volatiles of *Gutierezia sarothrae* (broom snakeweed, snakeweed) were isolated from ground, composite tissues by steam distillation and by solid phase microextraction (SPME), then separated and analyzed by gas chromatography with mass spectral and flame ionization detection. Compounds detected varied in quantity between isolation protocols. In the oil, cryptone (6.4%) and β-eudesmol (5.9%) were the only compounds comprising more than 5% of the chromatographic peak area. In samples prepared by solid-phase microextraction, limonene (10.4%), β-pinene (9.6%), β-eudesmol (8.0%), sabinene (7.8%), cryptone (6.5%), α-pinene (5.5%) and o-cymene (5.2%) accounted for 53% of the extracted volatiles. The results revealed a complex volatile composition from which unique compounds may still be identified.

Key Word Index

Gutierezia sarothrae, broom snakeweed, Asteraceae, essential oil composition, limonene.

Introduction

Gutierrezia sarothrae (Pursh) Britt et.Rusby (snakeweed, broom snakeweed, terpentine bush) is a suffrutescent perennial found in rangelands throughout much of western North America. Because this plant spreads quickly on disturbed or drought-stressed rangelands and is toxic to domestic livestock, it is often considered a noxious weed. Snakeweed poultices have traditionally been used as externally applied remedies for flesh wounds, bee stings, snake bites (1), and rheumatism (2). Contemporary herbalists continue to sell tinctures and teas containing snakeweed.

Numerous studies have been directed towards identification and characterization of toxic components and developing strategies to control or eliminate snakeweed on agricultural rangelands. Significant characterizations of *Gutierrezia* flavonoids have been conducted (3-6). Diterpenes (7) and toxic saponins (8) have been identified, and novel chemicals have been described (6). However, in previous analyses of snakeweed volatiles only 17 compounds have been positively identified (9,10). Our objective is to provide a detailed profile of snakeweed essential oil.

Experimental

Collection of plant material: Five study sites with snakeweed as either a dominant or subdominant component were selected on the USDA Agricultural Research Service Jornada Experimental Range, and adjoining New Mexico State University Chihuahuan Desert Rangeland Research Center in south-central New Mexico. Elevation, annual precipitation, harvest dates and GPS coordinates for each site appear in Table I. At each site, 10 *G. sarothrae* plants were randomly selected from each of five wandering-quarter transects spaced 30 m apart. Plants less than 15 cm tall, without flowers, greater than 75% (visually estimated) dead standing material, or with visible signs of infestation by *Crossidius pulchellus* or *Myrmex linneolata* (root boring insects which cause considerable damage to living tissue) were not selected, as these were judged to be immature or physiologically stressed, and therefore likely to exhibit atypical foliar chemistry. A total of 88 plants were used for essential oil analysis. Branches containing green leaves were clipped, placed in labeled Whirl-pak bags, sealed, transported to the lab under dry ice, and stored at -20°C. Voucher

*Address for correspondence

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Table I. Locations, elevations and mean annual precipitation of snakeweed collection sites

Site	Harvest Date	Location*		Elevation (m)	Mean precipitation (mm)		
		Northing	Easting				Precipitation History**
1	9/26/2000	3609975	334501	1329	238	126	1927-2000
2	10/1/2000	3602542	320639	1313	225	107	1970-2000
3	9/12/2000	3609428	325458	1330	224	122	1918-2000
4	9/17/2000	3609278	329135	1336	240	121	1965-2000
5	9/13/2000	3613284	327556	1340	220	115	1927-2000

*locations are reported in Universal Transverse Mercator (UTM) coordinates for zone 13;**precipitation history indicates the years for which precipitation data has been recorded at each site.

specimens are located in the Department of Animal and Range Science Herbarium located at New Mexico State University in Las Cruces, New Mexico.

Sample preparation: Frozen plant material was ground in a mortar and pestle under liquid nitrogen. Ground tissue was passed through a 2 mm stainless steel sieve. Equal amounts (0.5 g) of tissue from each plant were blended into a single sample. Dry matter was determined by drying triplicate, 2 g samples of the composite tissue at 100°C for 24 h. Aliquots of this mixture were extracted by steam distillation and by solid phase microextraction (SPME).

Volatile extraction: Steam distillation was performed with a 15 g aliquot of plant tissue as previously described (11). SPME was optimized for simultaneous extraction of snakeweed monoterpenes and sesquiterpenes while minimizing extraction of larger semi-volatiles (data not shown). Extractions were carried out in triplicate using 0.2 g of composited tissue in 4 mL screw cap vials lined with PTFE/silicon septa (Supelco). Vials were equilibrated at 30°C for 3 h, then exposed to a 100 µm PDMS SPME fiber (Supelco) for 30 m. Immediately following exposure, SPME fibers were injected into the appropriate GC for 5 min.

GC/MS analysis: Volatiles isolated by both methods were separated and analyzed using a Varian 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). He at approximately 1 mL/min was used as a carrier gas, with injector and transfer line temperatures set at 220°C and 260°C, respectively. Initial column temperature was 60°C, and a linear temperature increase of 3°C/min was programmed into each 65 min run. This is essentially the analysis method described by Adams (12). For the oil, three injections (200 ng, 400 ng, 600 ng) were made to facilitate identification of both high and low concentration volatiles. With SPME extractions, the process of exposing a fiber modifies the equilibrium of the sample vial. Therefore, only a single injection was made for each of three extractions. Isocratic blank runs for seven minutes at 260°C were conducted after each SPME injection to ensure absence of residual volatiles on the fiber. Spectra from each chromatogram were manually examined for purity and matched to available libraries using Magnum GC/MS System software Version 3.0 (Finnigan Corp.). Compounds were identified by comparing mass spectra and Kovats (13) retention indices (RIs) with literature data (12,14,15) or with standards.

GC-FID analysis: Semi-quantitative analysis was performed by injecting samples into 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 He flow rate were identical to those used for GC/MS analysis. Injector temperature was 250°C.

Results and Discussion

Dry matter and oil yields: Dry matter accounted for 39% of the fresh plant material. Steam distillation isolated 5.2 mg of oil per g of fresh plant material (13.3 mg/g DM).

Volatile composition: More than 250 peaks were observed in GC/MS total ion chromatograms of injected oil, and additional 75-90 peaks were detected in chromatograms from the SPME injections. The majority of these peaks could not be positively identified either because of coelution or because levels were so low that clear spectra could not be obtained. Compounds that were positively identified are shown in Table II, along with peak area percent compositions. As expected, percent compositions varied between the FID and total ion chromatograms (TIC). FID sensitivity is less affected by compound structure, making it more reliable for comparing relative amounts of different compounds. In a few cases, peaks that appeared spectrally pure did not match RIs and spectra of compounds present in our libraries. The RIs and major spectral masses of these unknowns are provided in Table III.

The complex array of volatiles observed in snakeweed complements what the literature suggests is an equally complex nonvolatile profile. Compounds described in Tables II and III make up 67.6% (steam distillation) to 89.1% (SPME) of the total FID chromatograms, depending on the type of isolation used.

The most abundant (comprising over 5.0% of the total FID chromatograms) compounds varied between extraction protocols. In the oil, cryptone (6.4%) and β-eudesmol (5.9%) comprised only 14.3% of the oil. No other compounds exceeded 5.0% of the chromatographic peak area. In SPME, limonene (10.4%), β-pinene (9.6%), β-eudesmol (8.0%), sabinene (7.8%), cryptone (6.5%), α-pinene (5.5%) and o-cymene (5.2%) accounted for 53.0% of the extracted volatiles.

In previous work, geraniol (53.8%) and γ-humulene (12.2%) were isolated as major components of *G. sarothrae* leaves and

Table II. Compounds identified by retention index (RI) and 70 eV mass spectra in composite samples of broom snakeweed

Compound	RI	FID area %		TIC area %	
		SPME	oil	SPME	oil
tricyclene	928	t	ND	t	t
α-thujene	932	0.3	ND	0.4	0.5
α-pinene[m]	940	5.5	0.4	9.4	5.8
camphene	955	0.4	0.1	0.7	0.4
thuja-2,4(10)diene	960	0.2	0.2	0.1	0.3
sabinene	978	7.8	0.4	7	2.9
β-pinene[e]	981	9.6	0.7	8.7	5.6
6-methyl-5-heptene-2-one	986	t	ND	ND	0.1
myrcrene[m]	992	0.6	0.2	1.5	0.7
α-phellandrene	1006	0.9	0.3	2.4	1.6
δ-3-carene	1013	0.7	0.2	1.5	0.8
α-terpinene	1019	0.1	0.2	t	0.9
para-cymene	1024	0.3	0.2	0.7	0.7
ortho-cymene	1027	5.2	2.5	7.8	7.9
limonenee	1033	10.4	2.4	13.4	9
Z-β-ocimene	1041	ND	ND	t	t
benzene acetaldehyde	1044	t	ND	t	0.1
E-β-ocimene	1052	0.2	0.2	0.5	0.4
γ-terpinene	1062	0.2	0.6	0.1	1.6
cis-sabinene hydrate	1070	1.4	0.2	0.6	0.3
para-mentha-2,4(8)-diene	1090	0.3	0.5	0.6	1.1
linalool[m*]	1099	0.1	t	0.1	t
1,3,8-para-menthatriene	1113	0.1	ND	t	t
trans-thujone	1114	0.1	ND	t	0.1
dehydro-sabina ketone	1119	0.6	0.1	0.1	0.1
trans-para-mentha-2,8-dien-1-ol*	1123	0.4	0.7	0.1	1
α-campholenal	1127	ND	0.4	0.2	0.6
nopinonee	1138	0.6	0.4	0.3	0.9
trans-pinocarveole	1141	2.9	1.9	1.2	3.3
cis-verbenol[m]	1143	ND	ND	ND	0.5
trans-verbenol[m]	1147	1.4	1.3	0.7	1.6
sabina ketone	1158	1.3	1.1	0.7	1.7
pinocarvone[e]	1164	1.5	0.8	1.2	1.3
borneol	1168	0.5	0.4	0.3	1.8
terpinen-4-ol	1179	1.1	3.6	0.6	4.6
meta-cymen-8-ol	1182	0.2	0.8	0.1	0.9
cryptone	1185	6.5	6.4	2.4	8.1
verbenone[e,m]	1207	1.4	1.5	0.8	1.9
trans-carveol	1219	0.5	1.2	0.1	1.2
cis-carveol	1231	0.1	0.3	t	0.2
cumin aldehyde	1240	1.5	2	0.5	2.4
carvone	1244	0.9	1.3	0.4	1.4
E-2-decenal	1263	0.1	0.3	0	0.1
p-menth-1-en-7-al	1274	0.1	0.8	0.1	0.9
trans-carvone oxide	1277	0.1	0.1	0.1	0.1
α-terpinen-7-al	1282	0.1	0.5	0	0.2
borneol acetate[e]	1286	3.7	2.8	3.6	4.5
para-cymen-7-ol	1289	0.2	1	t	0.7
perilla alcohol	1297	0.2	0.1	t	0.1
carvacrol	1298	0.1	0.1	t	0.1
presilphiperfol-7-ene*	1334	0.3	0.9	0.2	1.2
7-epi-silphiperfol-5-ene	1345	0.3	0.4	0.3	0.3
α-cubebene	1352	0.1	0.5	0.1	0.3
cyclosativene	1369	0.3	0.4	0.5	0.4
α-ylangene	1373	0.1	0.3	0.1	0.1
silphiperfol-6-ene	1376	ND	2.1	ND	t
α-copaene	1377	0.8	0.3	2.1	2.2
β-maaliene	1381	0.3	ND	0.3	0.2
β-bourbonene	1385	ND	0.2	ND	t
isocomene	1387	0.2	0.1	0.2	0.2

Table II. continued

Compound	RI	FID area % SPME	FID area % oil	TIC area % SPME	TIC area % oil
β-cubebene	1391	1.1	0.1	2.7	0.3
β-elemene	1392	0.2	0.3	ND	0.2
β-isocomene	1405	0.2	0.2	0.1	0.2
β-caroyophyllene[m]	1420	3.2	2.3	4.8	2.6
β-copaene	1430	0.2	0.3	0.4	0.3
α-*trans*-bergamotene	1438	0.1	0.2	0	0.1
Z-β-farnesene	1446	0.1	0.1	t	t
sesquisabinene	1459	0.1	0.2	0.1	0.1
γ-gurgenene	1474	ND	0.1	0.1	t
trans-cadina-1,(6),4-diene	1475	0.2	0.4	0.2	0.3
β-bisabolene	1509	0.6	0.5	0.6	0.7
elemol	1551	0.1	0.3	t	t
E-nerolidol	1565	0.1	0.6	t	0.4
prenopsan-8-ol	1574	0.2	0.4	t	0.1
himachalene epoxide	1577	1.5	1.2	0.1	0.4
spathulenol	1578	ND	ND	ND	0.5
caryophyllene oxide	1583	ND	2.4	0.8	1.8
gleenol	1585	t	0.2	t	t
guaiol*	1597	t	0.8	t	0.1
humulene epoxide II	1609	0.4	0.5	t	0.3
β-himachalene oxide	1612	ND	1.1	0.1	0.9
silphiperfol-6-en-5-one	1623	t	ND	t	0.1
1-epi-cubenol	1629	t	0.4	t	0.2
γ-eudesmol	1633	t	0.3	t	0.1
cubenol	1643	t	1	t	0.4
β-eudesmol	1651	8	5.9	0.1	2.5
neo-intermedeol	1656	ND	0.8	t	0.4
bulnesol	1668	ND	0.7	t	0.3
epi-α-bisabolol	1684	ND	1.6	t	0.7
6S-7R-bisabolone	1747	ND	0.3	t	0.1

t = trace; compounds previously described by Epstein and Seidel (1989) are indicated with [e]; compounds previously described by Molyneux, Stevens, and James (1980) are indicated with [m]; *=compounds with low spectral fit scores (850-950) determined using Magnum™ V. 3.0 software; all other identified compounds have fit scores > 950

Table III. Kovats (13) retention indices (RI) and mass spectra of unidentified compounds*

RI	FID peak area %	Mass spectra at 70 eV
881	0.2	41 (100), 42 (42), 43 (43), 44 (12), 55 (18), 56 (16), 57 (11)
976	0.1	41 (100), 42 (58), 43 (52), 44 (52), 51 (13), 77 (13), 91 (44), 119 (57)
1106	0.1	41 (71), 42 (13), 43 (100), 44 (15), 45 (10), 50 (11), 51 (20), 53 (17), 55 (22), 65 (15), 67 (39), 69 (30), 70 (16), 79 (33), 81 (19), 82 (10), 91 (57), 92 (23), 93 (15), 95 (20), 97 (14), 109 (28), 19 (10)"
1249	0.2	42 (100), 42 (32), 43 (51), 43 (81), 44 (36), 51 (11), 53 (18), 55 (94), 67 (18), 69 (22), 79 (13), 81 (17), 82 (10), 83 (39), 97 (15)"
1252	0.1	41 (26), 43 (14), 50 (17), 63 (15), 65 (11), 77 (45), 78 (27), 79 (86), 80 (13), 91 (54), 92 (10), 103 (13), 105 (67), 106 (37), 115 (16), 117 (23), 119 (25), 133 (10), 135 (100), 149 (11)"
1329	0.5	41 (36), 43 (32), 51 (13), 67 (10), 77 (26), 79 (100), 91 (54), 92 (47)
1554	0.2	41 (100), 42 (13), 43 (59), 44 (52), 45 (21), 51 (11), 53 (17), 55 (32), 65 (12), 67 (29), 69 (18), 77 (24), 79 (59), 81 (20), 91 (40), 93 (29), 105 (17), 106 (10), 107 (17), 109 (11), 121 (12), 149 (12)

*only ions consisting of 10% of the base peak or more are listed; putative mass ions are not indicated since they have not been confirmed with soft ionization

stems collected in Eunice, New Mexico during the spring of 1979 (10). Seven of the nine compounds reported by Molyneux et al. were present in our samples (Table II, identified with [m]). Two reported compounds, geraniol and γ-humulene, could not be identified in our samples.

cis-3-Pinene-2-ol (up to 1.9%) and myrentol (up to 11.3%), were reported in snakeweed oil from whole plants collected in Muskrat Canyon, Utah (9), along with α-pinene, β-pinene, limonene, *trans*-pinocarveol, nopinone, *trans*-verbenol, pinocarvone, verbenone, and bornyl acetate. With the exceptions of myrentol and *cis*-3-pinene-2-ol, all of these compounds were detected in our samples. The Muskrat Canyon samples, like

ours, came from whole plants. Several collections were made in both spring and fall from 1983 to 1987. Compounds that were consistently reported in high percent compositions were β-pinene, α-pinene, and limonene. All of these compounds were abundant in our SPME extracts as well. Myrentol concentrations in the Muskrat Canyon samples ranged from 11.3% detected in fall of 1984 to only a trace detected in spring 1986.

With such spatial and temporal differences in collection sites, and with different harvest and isolation techniques, it is not surprising to see different profiles between studies. Significant isozyme and morphological variability between snakeweed populations (16,17), has been reported. Genetics, phylogeny, environmental factors, and even sampling and storage protocols can all contribute to differences in volatile expression patterns within a species. Our samples were collected during a drought. Growing season precipitation prior to sampling was less than half the mean for each of the collection sites.

We have previously compared results obtained using steam distillation to isolate shrub oils with results obtained by SPME (18). In general, SPME favors the extraction of monoterpenes, while steam distillation profiles reveal greater percentages of higher molecular weight compounds. This pattern is observed numerically in Table II. Drawbacks to steam distillation include the extensive time involved in sample preparation, and the possible introduction of oxidative byproducts. SPME is rapid, requires only small amounts of plant material and facilitates replication. In our experience, more compounds can be detected in SPME extractions as described above than in steam-distilled oils of the same plant material. However, quantitative yields per gram of plant material are difficult to determine from SPME extractions.

The 97 volatile compounds described in this paper represent the most comprehensive profile of snakeweed volatiles reported. Nonetheless, the abundance of unidentifiable peaks in our chromatograms demonstrate that more work is required to fully define this complex mixture. We believe that the wealth of existing chemical, toxicological, ethnobotanical, and ecophysiological data available for *G. sarothrae* provide an extensive foundation from which to study the bioactive and pharmaceutical properties of its extracts. In addition, the continued use of this plant by the general public warrants investigation of its utility and safety.

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