Volatile Constituents of the Aerial Parts of *Salvia apiana* Jepson

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Abstract

Volatile constituents of the aerial parts of fresh white sage (*Salvia apiana* Jepson) were isolated by extraction with diethyl ether followed by high vacuum distillation with a solvent assisted flavor evaporation (SAFE) apparatus. The isolated volatiles were analyzed by GC and GC/MS. A total of 84 constituents were identified (constituting 95.1% of the total area), 11 of which were tentatively identified. The volatiles were characterized by a high content of hydrocarbon and oxygenated monoterpenes. The major constituents identified were 1,8-cineole (34.5%), camphor (21.7%), β-pinene (7.4%), α-pinene (6.4%), δ-3-carene (6.4%), camphene (3.9%), limonene (3.5%), myrcene (3.2%), and terpinolene (1.3%).

Key Word Index

Salvia apiana, white sage, Lamiaceae, essential oil composition, 1,8-cineole, camphor, 1,3,5-undecatriene isomers, 1,3,5,8-undecatetraene isomers.

Introduction

Salvia apiana Jepson is one of approximately 900 worldwide species of *Salvia* found in the Lamiaceae family (1). It generally grows below 1500 m in Baja California, in the South Coast (SCo), Transverse Ranges (TR), and Peninsular Ranges (PR) sub-regions of southwestern California as well as in the western edge of the Desert Province (DR) in the southeastern portion of California (1). The plant has been used to treat chest colds, coughs, sore throats, systemic poison oak rashes and acute candidal vaginitis, and has been widely used by natives (2,3) and in traditional Chumash healing (4). The leaves were eaten, smoked and used in sweat baths by Cahuilla Indians to treat upper respiratory infections (3). Four new diterpenes, 6,7-didehydroferruginol; 6,7-didehydrosempervirol; 16-hydroxy-6,7-didehydroferruginol; 11,12,16-trihydroxy-20(10→5) abeo-abieta-1(10),6,8,11,13-pentaene, two new diterpene quinones, 16-hydroxyroyleanone and 6-deoxo-5,6-didehydrolanugon Q as well as the known compounds, ferruginol, miltiodiol, cryptanshinone, lanugon Q and salvicanol have been isolated and characterized from the roots of *S. apaina* (5).

α-Amyrin, oleanolic acid and ursolic acid were identified in the dried aerial parts of *S. apiana* (6). Dentali and Hoffmann (7) identified two abietane acids, 16-hydroxycarnosic acid and carnosic acid in the leaves of *S. apiana*. Luis and co-workers (8,9) identified the new C_{23} terpenoids, 14-hydroxy-7-methoxy-11,16-diketo-apian-8-en-(22,6)-olide, 7-methoxy-11,16-diketo-apian-8,14-dien-(22,6)-olide, and 13,14-dioxo-11-hydroxy-7-methoxy-hassane-8,11,15-trien-(22,6)-olide along with the known diterpenes, 16-hydroxycarnosic acid, 16-hydroxycarnosol, 16-hydroxyrosmanol, 16-hydroxy-7-methoxyrosmanol, rosmanol, 7-epirosmanol and salvicanol in the aerial parts of *S. apiana*. The volatile constituents from *S. apiana* Jepson leaves have been shown to inhibit the root growth of *Cucumis sativus* and *Avena fatua* seedlings (10). It was postulated the volatiles may be deposited when dew condenses on the seedlings in the field though the active constituent(s) was not characterized.

While the composition of volatiles from the essential oils of numerous *Salvia* species have been reported (11–18) there is only limited knowledge of the volatile constituents in white sage (19–21). The aim of this study was to provide a more

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Table I. Volatile constituents (%) of the aerial parts of *Salvia apiana* Jepson

Constituent	I_{DB-1} exptl	I_{DB-1} ref	% area[a]	Constituent	I_{DB-1} exptl	I_{DB-1} ref	% area[a]
(Z)-3-hexenol	843	834	tr.[b]	*cis*-piperitol	1177	1175	tr.
hexanol	860	860	tr.	(E)-2-octenyl acetate	1182	1191	tr.
3-methylbutyl acetate	865	866	tr.	*trans*-piperitol	1185	1185	tr.
3-methyl-3-butenyl acetate	871	861	tr.	(Z)-3-hexenyl isovalerate	1220	1219	tr.
3-methyl-2-butenyl acetate	909	902	tr.	piperitone	1224	1224	tr.
tricyclene	915	918	0.1	hexyl isovalerate	1228	1228	tr.
α-thujene	923	922	0.3	geranial	1241	1241	tr.
α-pinene	930	929	6.4	bornyl acetate	1265	1268	0.2
camphene	940	941	3.9	2-undecanone	1270	1273	tr.
sabinene	965	964	0.2[c]	(Z)-3-hexenyl tiglate	1300	1300	tr.
β-pinene	967	968	7.4	4-methoxyacetophenone	1303	1302	tr.
2-pentylfuran	981	977	tr.	hexyl tiglate	1310	1310	tr.
myrcene	985	981	3.2	eugenol	1323	1327	tr.
(Z)-3-hexenyl acetate	990	986	tr.	neryl acetate	1343	1342	tr.[g]
(p-menth-1(7),8-diene)[d]	992	(1004)	0.1	α-cubebene	1343	1347	tr.
α-phellandrene	993	996	0.4	geranyl acetate	1360	1360	tr.
δ-3-carene	999	1004	6.3	(Z)-jasmone	1361	1365	tr.
α-terpinene	1006	1008	0.2	α-ylangene	1364	1370	tr.
p-cymene	1008	1010	tr.	α-copaene	1368	1374	0.1
1,8-cineole	1016	1018	34.5[c]	α-gurjunene	1399	1408	0.1
limonene	1018	1020	3.5	β-caryophyllene	1406	1418	1.0
(Z)-β-ocimene	1030	1026	0.7	geranylacetone	1422	1427	tr.
(E)-β-ocimene	1041	1037	0.3	guaia-6,9-diene	1431	1437	0.2
γ-terpinene	1049	1048	0.4	(selina-4(15),6-diene)[f]	1435	(1450)	tr.
cis-sabinene hydrate	1052	1051	0.2	α-humulene	1440	1449	0.1
trans-linalool oxide A furanoid	1057	1056	tr.	(7αH,10βH-cadina-1(6),4-diene)[f]	1461	(1472)	tr.
fenchone	1063	1065	tr.	γ-muurolene	1463	1469	0.1
2-nonanone	1073	1069	tr.	α-amorphene	1466	(1477)	tr.
terpinolene	1077	1077	1.3	bicyclogermacrene	1482	1489	0.1
(*trans*-sabinene hydrate)[d]	1079	(1098)	0.2	α-muurolene	1486	1492	0.1
linalool	1090	1083	0.2	β-bisabolene	1496	1500	0.2[c]
campholene aldehyde	1097	1103	tr.	γ-cadinene	1496	1505	0.2
(*trans*-p-menth-2-en-1-ol)[d]	1108	(1136)	tr.	calamenene*	1500	1508	tr.
camphor	1112	1118	21.7	δ-cadinene	1507	1514	0.3
ipsdienol	1125	1126	tr.	(*trans*-cadina-1,4-diene)[f]	1516	(1523)	tr.
borneol	1144	1147	0.2	(α-cadinene)[f]	1522	(1534)	tr.
terpinen-4-ol	1157	1159	0.2	(E)-α-bisabolene	1528	1532	tr.
1-(E,Z)-3,5-undecatriene	1165	1163	0.2	selina-3,7(11)-diene	1531	1537	tr.
α-terpineol	1168	1170	tr.	germacrene B	1540	1550	0.2
(1,3,5,8-undecatetraene)[e]	1172		tr.	(T-cadinol)[f]	1615	(1633)	tr.
1-(E,E)-3,5-undecatriene (1,3,5-undecatriene)[e] + 1-(E,Z,Z)-3,5,8-undecatetraene	1173 1174	1172 1175	tr. tr.	(6α-hydroxygermacra-1(10),4-diene)[f]	1664	(1687)	0.1

[a] Peak area percentage of total FID area (assuming all response factors of 1); [b] tr. represents a % area < 0.1%; [c] peak area of this constituent and the following constituent were calculated on the basis of the GC/MS total ion chromatogram; [d] Tentative identifications (in parentheses) assigned based on mass spectra and I^{DB-5} reported in Adams (2007); [e] Tentative identifications (in parentheses) assigned based on mass spectra reported in Wiley Registry of Mass Spectral Data, 8th Edition; [f] Tentative identifications (in parentheses) assigned based on mass spectra and I^{DB-5} reported in MassFinder 3 (Dr. Hochmuth Scientific Consulting, Hamburg, Germany); [g] This constituent and the next eluting constituent were resolved by GC/MS but were not separated by GC-FID; * correct isomer not identified.

comprehensive knowledge of the volatiles in the aerial parts of *S. apiana* Jepson.

Experimental

Plant material: Fresh leaves and flowering tops of *S. apiana* Jepson were collected in the UC Davis Botanical Gardens in June 2007. The samples were prepared the same day that they were picked. A voucher specimen was deposited in the Jepson Herbarium, University of California, Berkeley, CA.

Chemicals: Diethyl ether was freshly distilled through a 60 cm long Pyrex column packed with glass helices and stored in the dark after addition of 1–2 ppm of antioxidant 330 (1,3,5-trimethyl-2,4,6-tris-[3,5-di-tert-butyl-4-hydroxybenzyl]-benzene; Ethyl Corporation, Richmond, VA).

Extraction of volatiles: The plant material (95 g) was crushed with a mortar and pestle under liquid nitrogen. The material was divided into equal portion and added to two 250 mL Pyrex glass bottles with Teflon lined screw caps. Approximately 125 mL of ether was added to each bottle. The bottles were covered with aluminum foil and were sonicated in an ultrasonic bath for 15 min. The bottles were shaken throughout the day every 2 h and allowed to stand overnight. The dark

green extract was filtered through pre-rinsed (ether) filter paper (ED fluted filter paper, grade 513, size 24 cm, Eaton-Dikeman, Mount Holly Springs, PA). The extract was dried overnight over anhydrous sodium sulfate (previously heated to 150°C for several hours to remove volatiles). The extract was subjected to high vacuum distillation using a solvent assisted flavor evaporation (SAFE; 22) apparatus. The SAFE apparatus was heated to 40°C with a circulating water bath and the extract was added to the dropping funnel of the apparatus. The distillation flask (500 mL) was heated to 40°C in a water bath. The receiving flask for the distillate and the safety-cooling trap of the SAFE apparatus were cooled with liquid nitrogen. The SAFE apparatus was connected to a high vacuum pump (< 0.01 Pa) and then the mixture in the dropping funnel was added in small aliquots into the distillation flask over 20 min. The distillate was concentrated using a Vigreux column (15 x 1 cm) and water bath at 40°C. The extract (0.6637 g) was used for GC and GC/MS analyses.

Gas chromatography: A Hewlett-Packard (Avondale, PA) 6890 gas chromatograph equipped with a flame ionization detector (FID) was used. A 60 m X 0.32 mm DB-1 (d_f = 0.25 µm; J&W Scientific, Folsom, CA) fused silica capillary column was employed. The oven temperature was programed from 30°C (4 min isothermal) to 200°C at 2°C/min (final hold was 25 min). Split injections (1:20) were used. Helium was used as the carrier gas at a linear velocity of 38.3 cm/s (30°C).

Gas chromatography/mass spectrometry (GC/MS): The GC/MS system consisted of an Agilent Technologies 6890 gas chromatograph coupled to an Agilent Technologies 5973 Network MSD (Agilent Technologies, Palo Alto, CA). A 60 m X 0.25 mm DB-1 MS fused silica capillary column was used (d_f = 0.25 µm). The GC oven was programed from 30°C (4 min isothermal) to 200°C at 2°C/min (final hold was 35 min). Helium was used as the carrier gas at a headpressure of 22 psi. The injector, transfer line, ion source and quadrupole temperatures were 180°C, 200°C, 170°C and 130°C, respectively. The mass spectrometer was operated in the electron impact mode with an ionization voltage of 70 eV. A scan range of *m/z* 35–320 at 4.94 scans/s was employed.

Identification of volatiles: Volatile constituents were identified by comparing the component's mass spectrum and experimental retention index (I) with that of an authentic reference standard. The retention system proposed by Kováts (23) was utilized. When standards were not available, tentative identifications were assigned based on mass spectra and retention indices reported in Wiley Registry of Mass Spectral Data, 8th Edition (John Wiley & Sons, Inc., Hoboken, NJ), NIST/EPA/NIH Mass Spectral Library 2005 (U.S. Department of Commerce), MassFinder 3 (Dr. Hochmuth Scientific Consulting, Hamburg, Germany) and Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry, 4th Edition (24).

Results and Discussion

Aerial portions of *S. apiana* Jepson were extracted with diethyl ether and the volatiles were isolated by high vacuum distillation using a SAFE apparatus. GC analysis of the extract (0.6637 g) revealed that volatiles constituted 57.5% (0.3816 g) while ether made up 42.5% (0.2821 g). The yield of volatiles from the sample was 0.4%. A total of 84 constituents were identified (constituting 95.1% of the total area), 11 of which were tentatively identified. The major constituents identified were 1,8-cineole (34.5%), camphor (21.7%), β-pinene (7.4%), α-pinene (6.4%), δ-3-carene (6.4%), camphene (3.9%), limonene (3.5%), myrcene (3.2%), and terpinolene (1.3%). Emboden and Lewis (20) reported similar percentages of 1,8-cineole (39.5–46.6%), combined camphor and borneol (30.6–40.1%), β-pinene (6.7–7.6%), α-pinene (5.5–6.2%), camphene (3.9–4.9%) and limonene (3.3–5.1%) in *S. apiana* subsp. *apiana* oil. A more recent study on *S. apiana* oil (21) found similar levels of β-pinene (9.1%), α-pinene (9.0%), and limonene (2.0%) but lower levels of δ-3-carene (1.3%), camphene (0.4%) and camphor (2.1%). 1,8-Cineole was also reported as the main constituent though its percentage (71.6%) was higher in the previous investigation (21). 1,8-Cineole has been reported to be useful for the treatment of brochial asthma, cough, and liver failure induced by endotoxemic shock (25–27). This monoterpene oxide has been shown to possess gastroprotective activity, an effect related to both its antioxidant activity and its lipoxygenase inhibitory effects (28). Juergens and co-workers (29) demonstrated that 1,8-cineole was a strong inhibitor of TNF-α and IL-1β production in stimulated lymphocytes and monocytes. They also showed that 1,8-cineole at known therapeutic blood concentrations had inhibitory effects on the chemotactic cytokine of IL-8 and IL-5. The reduction of cytokine production suggested an anti-inflammatory mode of action and consequently inhibition of cytokine induced airway mucus hypersecretion rather than simple secretolytic activity. Isolated monoterpenes such as 1,8-cineole may offer a new opportunity for initial and long-term treatment of asthma and chronic obstructive pulmonary disease (COPD).

Salvia fructicosa oil and its major compounds, thujone and 1,8-cineole, showed relatively low antimicrobial activity against eight bacterial strains, *Escherichia coli* (NCIMB 8879 and NCIMB 12210), *Pseudomonas aeruginosa* (NCIMB 12469), *Salmonella typhimurium* (NCIMB 10248), *Staphylococcus aureus* (NCIMB 9518 and NCIMB 8625), *Rhizobium leguminosarum* (NCIMB 11478), and *Bacillus subtilis* (NCIMB 3610), while camphor was exhibited almost no activity against the bacteria tested (30). *Salvia fructicosa* oil and 1,8-cineole, camphor and thujone exhibited cytotoxic activity against African Green Monkey kidney (Vero) cells and high levels of virucidal activity against herpes simplex virus 1 (30). Pitarokili and co-workers (15) tested the antifungal activity of 1,8-cineole and camphor (the main constituents identified in *S. fruticosa* oil) against five phytopathogenic fungi, *Fusarium oxysporum* f. sp. *dianthi*, *Fusarium proliferatum*, *Fusarium solani* f. sp. *cucurbitae*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum*. Camphor showed moderate activity against *S. sclerotiorum* and *R. solani* but displayed lower activity against the three *Fusarium* species. 1,8-Cineole exhibited only slight activity against the five fungal species. The oil of *S. fruticosa* exhibited higher antifungal activity than camphor which led the researchers to conclude that other components exert direct activity or possibly a synergistic effect with camphor. Three previously reported *S. apiana* constituents, cymene, α-pinene oxide and β-caryophyllene oxide, were not detected in this study (21).

These authors also did not detect α- and β-thujone which are major constituents in *Salvia officinalis* L. oil (12,14,16). To the best of these authors' knowledge this is the first time that 1,3,5-undecatriene and 1,3,5,8-undecatetraene isomers have been reported in *Salvia* species.

The potent odor character of 1-(E,Z)-3,5-undecatriene and 1-(E,Z,Z)-3,5,8-undecatetraene has been described by Berger et al. (31). The configuration of the double bond in the C-5 position is crucial as the corresponding isomers, 1-(E,E)-3,5-undecatriene and 1-(E,Z,Z)-3,5,8-undecatetraene have odor thresholds 10^6 and 10^4 times higher, respectively (31). 1-(E,Z)-3,5-Undecatriene has a balsamic, spicy, pinewood odor while 1-(E,Z,Z)-3,5,8-undecatetraene has a similar though more fruity odor (31). Sesquiterpene hydrocarbons were identified for the first time in *S. apiana,* though they have been reported in other *Salvia* species (13,17,18). The most abundant sesquiterpenes were β-caryophyllene (1.0%), δ-cadinene (0.3%), germacrene B (0.2%), guaia-6,9-diene (0.2%), β-bisabolene (0.2%), γ-cadinene (0.2%), α-copaene (0.1%), α-gurjunene (0.1%), α-humulene (0.1%), γ-muurolene (0.1%), bicyclogermacrene (0.1%), and α-muurolene (0.1%).

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