lene (10.1–14.2%). The leaf oils, which were characterized by a high content of α-copaene (14.2% against 1.0% and 0.9% for the bark and root oils), present the most complex chemical composition, with 6.4% of oxygenated sesquiterpenes (against 2.7% and 2.1% for the bark and root oils).

On another hand, no significant antiradical activity of these essential oils could be observed according the DPPH method; finally, considering the lack of inhibitory effect on the soybean lipoxygenase activity, the use of this plant as an anti-inflammatory drug should not be recommended in traditional medicine.

Acknowledgments

The authors are grateful to the Government of Benin and the French Cooperation for financial support.

References

1. P. Bamps, *Répartition de* Pentadesma butyracea *en Afrique*. Bulletin du Jardin Botanique National de Belgique, **41**, 430–432 (1971).
2. A.K. Natta, *Ecological assessment of riparian forests in Benin: Phytodiversity, phytosociology and spatial distribution of tree species*. pp. 73–88, Ph.D. University of Wageningen, Wageningen, Netherlands (2003).
3. J. Hutchinson and J.M. Dalziel, *Flora of West Tropical Africa*. 2nd Edn., Vol. 1, Part 1, p. 295 (1954).
4. B. Sinsin and A.T. Sinadouwirou, *Valorisation socio-économique et pérennité du* Pentadesma butyracea *Sabine en galeries forestières au Bénin*. Cahiers d'études et de recherches francophones / Agriculture, **12**, 75–79 (2003).
5. D.J. Mabberley, *The Plant-Book*. Cambridge University Press, Cambridge, UK (1987).
6. L. Dencausse, H. Nsourankoua, J. Artaud and J.L. Clamou, *Comparaison des compositions lipidiques des beurres de Pentadesma et de Karité*, OCL 2, 143–147 (1995).
7. O. Courtin, *Cosmetic preparation to retard the ageing skin*. Eur. Pat. Appl., CODEN:EPXXDW EP 180505 A1 19860507, p. 13 (1986).
8. A. Raponda-Walker and R. Sillans, *Les plantes utiles du Gabon*. p. 614, Paul Lechevalier, Paris, France (1961).
9. D.K. Abbiw, *Useful plants of Ghana*. S. 154–157, Intermediate Technology Publications and the Royal Botanic Gardens Kew, London, UK (1990).
10. F.P. Tchobo, A.K. Natta, B. Barea, N. Barouth, G. Piombo, M. Pina, P. Villeneuve, M.M. Soumanou and D.C.K. Sohounhloue, *Characterization of* Pentadesma butyraceae *Sabine butters of different production regions in Benin*. J. Amer. Oil Chem. Soc., **84**, 755–760 (2007).
11. S.P. Gunasekera, K. Sivapalan, M.U.S. Sultanbawa and O.W. Ollis, *Chemical investigation of ceyloneses plants. Part 21. Extractives of* Pentadesma butyracea *Sabine.* J. Chem. Soc. Perkin Trans 1, **1**, 11–14 (1977).
12. D. Joulain and W.A. König, *The Atlas of Spectral Data sesquiterpene hydrocarbons.* E.B. Verlag, Hamburg, Germany (1998).
13. R.P. Adams, *Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectrometry.* Allured Publ. Corp., Carol Stream, IL (2001).
14. A. Mellors and A.L. Tappel, *The inhibition of mitochondrial peroxidation by ubiquinone and ubiquinol*. J. Biol. Chem., **241**, 4353–4356 (1966).
15. A. Agnaniet, C. Menut and J.M. Bessière, *Aromatic Plants of Tropical Central Africa. Part XLIX. Chemical composition of essential oils of the leaf and rhizome of* Aframomum giganteum *K. Schum from Gabon*. Flav. Fragr. J., **19**, 205–209 (2004).
16. G.A. Alitonou, F. Avlessi, D.K. Sohounhloue, H. Agnaniet, J.M. Bessière and C. Menut, *Investigations on the essential oil of* Cymbopogon giganteus *from Benin for its potential use as an anti-inflammatory agent*. Intern. J. Aromather., **16**, 17–41 (2006).

Composition of *Ceanothus gregii* Oil as Determined by Steam Distillation and Solid-Phase Microextraction

Mary E. Lucero,* Rick E. Estell and Ed Fredrickson,
USDA-ARS Jornada Experimental Range, P.O. Box 30003, MSC 3JER, Las Cruces, NM 88003-8003

Abstract

Ceanothus gregii Gray was collected from the Jornada Experimental Range in south central New Mexico. Current year's growth was collected from ten plants found within an approximate 50 m radius of N32°40.605' and W106°33.486'at an altitude of 1,741 m 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 the same plants was also examined using solid-phase microextraction (SPME) with a 100 μm polydimethylsiloxane fiber. Mass spectra and retention indices were used to identify 41 previously described compounds. Methyl salicylate (16.8%), hexanal (11.8%) and decanal (7.0%) were the major identified compounds.

Key Word Index

Ceanothus gregii, Rhamnaceae, essential oil composition, methyl salicylate, hexanal.

*Address for correspondence

Received: January 2008
Revised: March 2008
Accepted: August 2008

Introduction

Ceanothus greggii Gray, commonly known as desert ceanothus or red root, is a woody shrub of the family Rhamnaceae found in desert shrub communities throughout the southwestern United States and northern Mexico. The genus *Ceanothus* contains 54 other species endemic to North America (1). Species within the genus have been described with respect to genetic diversity (1,2), wildlife forage value (2,3) and traditional medicinal uses (4). *Ceanothus* species, including *C. greggii*, have been used by herbalists to treat pharyngitis, tonsillitis, headaches, splenitis, and red blood cell clumping (5). Little attention has been given to the essential oil composition of the genus. No published essential oil compositions were found for *C. greggii*.

Experimental

Plant material was collected from the USDA-ARS Jornada Experimental Range in southern New Mexico, at an altitude of 1,741 m above sea level. Ten plants were randomly selected from within a 50 m radius of N32°40.605' and W106°33.486'.

Table I. Percentage composition of the oil and SPME headspace volatiles of *Ceanothus greggi*

		TIC Area %		FID Area %	
RI	**Compound**	**oil**	**SPME**	**oil**	**SPME**
797	hexanal	nd	0.1	11.8	10.7
835	isovaleric acid	t	0.1	0.1	0.2
868	hexanol	2.3	0.5	4.6	1.7
899	heptanal	0.5	2.9	nd	0.1
939	α-pinene	t	0.2	nd	1.0
970	heptanol	t	1.2	nd	0.0
981	β-pinene	t	1.3	nd	0.1
986	6-methyl-5-hepten-2-one	0.1	t	nd	0.3
991	dehydro-1,8-cineole	0.5	0.5	0.1	nd
992	myrcene	t	22.2	nd	4.9
1001	octanal	0.1	t	1.8	0.1
1012	δ-3-carene	t	t	nd	4.3
1031	limonene	0.1	0.4	2.1	8.6
1066	acetophenone	0.1	0.5	0.7	0.1
1072	octanol	t	0.2	t	nd
1075	m-cresol	1.2	0.7	t	0.2
1089	p-mentha-2,4(8)diene	0.5	t	t	0.1
1093	unknown[1]	0.1	0.3	3.4	t
1099	unknown[2]	3.4	0.1	2.4	0.1
1113	unknown[3]	4.8	0.1	2.4	t
1137	*cis*-p-mentha-2,4-dien-1-ol	0.2	t	t	0.1
1143	*cis*-verbenol	0.1	0.2	t	0.1
1147	*trans*-verbenol	0.6	1.2	t	t
1171	ethyl-benzoate	0.2	0.1	t	0.3
1178	terpinen-4-ol	1.2	0.1	t	0.9
1184	p-cymen-8-ol	4.2	0.2	t	6.2
1190	α-terpineol	0.2	0.1	t	0.1
1192	methyl salicylate	8.7	0.6	16.8	0.2
1204	decanal	0.7	t	7.0	t
1206	verbenone	0.8	t	0.2	0.1
1229	nerol	0.3	t	t	1.4
1245	ethyl phenylacetate	0.3	0.3	t	0.1
1256	geraniol	0.7	0.3	t	0.1
1262	(E)-2-decenal	0.5	0.1	t	t
1306	undecanal	0.5	t	t	t
1357	eugenol	0.2	0.2	t	0.3
1383	(E)-β-damascenone	1.6	0.1	1.0	0.1
1408	dodecanal	0.6	t	t	t
1485	(E)-β-ionone	0.3	t	t	0.9
1612	tetradecanal	0.1	t	t	0.1
1632	(Z)-3-hexenyl phenylacetate	0.1	0.5	t	0.1
1650	β-eudesmol	t	t	t	t
1761	benzylbenzoate	0.1	0.2	nd	nd
1827	isopropyl tetradecanoate	0.4	0.3	nd	nd

Compounds identified by retention index (RI) and 70eV mass spectra in steam distilled oil or SPME extracts of *Ceanothus greggii*; Compounds with peak areas that comprised less than 0.1% of the chromatogram are indicated with "t" for "trace"; "nd" indicates that a compound was not detected; [1]41(21), 43(14), 44(100), 45 (15), 50(28), 51(44), 77 (62), 92(11), 105(51); [2]41 (80), 42(12), 43(100), 53(15), 55(44), 67(24), 69(24), 71(49), 79(16), 80(30), 81(41), 91(10), 92(13), 93(44), 121(12); [3]41(100), 42(37), 43(42), 44(13), 50(14), 51(20), 53(22), 55(17), 63(11), 65(20), 67(34), 77(18), 79(34), 81(19), 91(32), 95(41), 109(89), 119(12).

Coordinates were determined using a Garmin GPS 12 personal navigator. Samples consisted of ten 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, SPME, and dry matter analyses were conducted. A voucher specimen identified as *Ceanothus greggii* (Gray) 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. Hydrodistillation was carried out as previously described (6), 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.

SPME was performed in triplicate by equilibrating 0.2 g of plant tissue (ground with liquid nitrogen as above) in 4 mL autosampler vials with PTFE/silicon septa for 4 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 30 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 (7,8) and with the authors' own MS library, developed using authentic standards.

To validate peak area percentages from the total ion chromatograms, 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 gradient rates, and He flow rate were identical to those used for the GC/MS analysis. The injector temperature was 250°C. Dry matter percentages were estimated by drying triplicate, 2 g samples of ground plant tissue at 100°C for 24 h.

Results and Discussion

Dry matter accounted for 84.2% of the tissue fresh weight. The steam distillate contained 0.38 mg of oil per g of dry matter. Table I shows the identities, retention indices (RI), and the percent compositions of 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 (7,8), and an MS library fit score greater than 950 as determined by Magnum 3.0 software. Peaks eluting prior to the first alkane used for determining retention indices (2.03 min and 2.18 min) accounted for 12.5% of the FID chromatogram. These peaks could not be observed in the total ion chromatograms (TIC) due to the 3 min solvent delay utilized. Because mass spectra were unavailable for these peaks, the compounds could not be identified. Four other compounds, with RIs of 1198, 1713, 2183, and >2200 accounted for 5.5%, 5.4%, 6.1%, and 4.8% of the FID chromatograms, respectively. However, these compounds produced small TIC peaks and poor spectra, hence they were omitted from Table I. Forty-one compounds were positively identified, accounting for only 46.3% of the steam distilled oil fraction. Low molecular weight compounds were more abundant in SPME than in steam distilled extracts. Methyl salicylate, an important plant defense compound with pharmaceutical applications (9,10) comprised 16.8% of the steam distilled oil. Hexenal (11.8%) and decanal (7.0%) were also identified as major compounds in the steam distilled oil.

References

1. T.M. Hardig, P.S. Soltis and D.E. Soltis, *Diversification of the North American shrub genus* Ceanothus *(Rhamnaceae): conflicting phylogenies from nuclear ribosomal DNA and chloroplast DNA*. Amer. J. Bot., **87**, 108–123 (2000).
2. M.W. Paschke, *Actinorhizal plants in rangelands of the western United States*. J. Range Management, **50**, 62–72 (1997).
3. M.W. Alldredge, J.M. Peek and W.A. Wall, *Nutritional quality of forages used by elk in northern Idaho*. J. Range Management, **55**, 253–259 (2002).
4. X.C. Li, L. Cai, and C.D. Wu, *Antimicrobial compounds from* Ceanothus americanus *against oral pathogens*. Phytochemistry, **46**, 97–102 (1997).
5. M. Moore, *Red root*. *Medicinal Plants of the Desert and Canyon West*. pp. 99–101, Museum of New Mexico Press, Santa Fe, NM (1989).
6. M.R. Tellez, R.E. Estell, E.L. Fredrickson and K.M. Havstad, *Essential oil of* chrysothamnus pulchellus *(Gray) greene ssp.* pulchellus. J. Essent. Oil Res., **10**, 201–204 (1998).
7. R.P. Adams, *Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy*. Allured Publ. Corp., Carol Stream, IL (1995).
8. R.P. Adams, *Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy*. Allured Publ. Corp., Carol Stream, IL (2001).
9. D.G. James and T.S. Price, *Field-testing of methyl salicylate for recruitment and retention of beneficial insects in grapes and hops*. J. Chem Ecol., **30**, 1613–1628 (2004).
10. V. Shulaev, P. Silverman and I. Raskin, *Airborne signalling by methyl salicylate in plant pathogen resistance*. Nature, 385 (1997).