J. Essent. Oil Res., **9**, 619-624 (Nov/Dec 1997)

RESEARCH REPORT

Essential Oil of *Flourensia cernua* DC.[1]

Mario R. Tellez, Rick E. Estell,* Ed L. Fredrickson and Kris M. Havstad
USDA-ARS, Jornada Experimental Range, Box 30003, Dept. 3JER, Las Cruces, NM 88003

Abstract

The leaf oil of *Flourensia cernua* DC. collected from the Jornada Experimental Range in southcentral New Mexico was analyzed. GC/MS and retention indices were used to identify 89 components. β-Eudesmol (24.5%), α-eudesmol (6.9%), limonene (6.6%), γ-eudesmol (4.6%), myrcene (3.8%), borneol (3.3%), and δ-3-carene (3.0%) were the major components of the steam distilled oil. In an ethanol extract of intact leaves, flourensadiol (44.6%), artemisia alcohol (5.5%), viridiflorol (2.7%), and borneol (2.0%) were the main components.

Key Word Index

Flourensia cernua, Asteraceae, essential oil composition, β-eudesmol, flourensadiol.

Introduction

Flourensia cernua DC. (tarbush, hojasen) is an aromatic deciduous shrub endemic to the Chihuahuan Desert of the southwestern United States and northern Mexico. Tarbush flowers are reported to be acutely toxic to livestock (1,2) when consumed in large amounts (approximately 1% of body weight per day). Additionally, its leaves and flower heads are sold in drug markets in Mexico (3) and local farmers' markets in the United States as a remedy for indigestion. Tarbush is increasing in dominance within the Chihuahuan Desert, and is currently the target of several studies involving its interactions with herbivores and of efforts directed towards determining biological controls for its management (4,5). A cursory profile of leaf surface terpenes was described by Estell et al. (6), and surface compounds have been associated with degree of herbivory in livestock (4). Various aspects of the chemical constituents of tarbush have been assessed: benzofurans and benzopyrans (7-9), long chain unsaturated hydrocarbons and thiophene derivatives (8), 2 sesquiterpenes (10-12), flavonoids (13-15), 2 fatty acids and a diglyceride (15). The oil of *F. cernua* has previously been isolated by Dominguez et al. (16). These workers established the presence of a free hydroxyl (IR frequency reported), a 'ketonic' compound and an 'insaturation' (no frequencies reported), and found no aromatic groups observable through NMR or IR spectra (no data given) in their product. However, they did not identify any of the components of *F. cernua*. To our knowledge, the oil composition of *F. cernua* has not been previously reported.

*Address for correspondence
[1]Mention of a trade name, proprietary product, or vendor does not constitute a warranty of the product by the USDA or imply its approval to the exclusion of other products or vendors that may also be suitable.

Received: November 1996

1041-2905/97/0006-0619$04.00/0—

Experimental

Plant materials were collected from an area heavily infested with tarbush on the Jornada Experimental Range. Ten plants were selected at random (excluding small juvenile plants) from each of three locations approximately 1.5 km apart. Ten leaders of current year's growth per plant were clipped, stored in plastic bags and immediately frozen using dry ice. Dry matter analyses and ethanol extracts were performed the same day. The remaining plant material was stored at –20°C until the steam distillations were performed (6-8 days). Dry matter (100°C/24 h) was determined in duplicate from ten whole midpoint leaves per plant. Ethanolic extracts were conducted by extracting 5 whole midpoint leaves per sample in duplicate with 5 mL of 100% ethanol (containing 5 ng/μL δ-2-carene as an internal standard) for 5 min with occassional shaking, followed by filtration through a fiberglass (Fisherbrand G8) filter. These leaves were then combined and further extracted with an additional 5 mL of 100% ethanol (containing 5 ng/μL δ-2-carene as an internal standard) and constant stirring for 5 days.

Steam distillations (17) were conducted in a 1000 mL flask fitted with a combination Claisen adapter/ distillation head which in turn was fitted with an insulated, pre-heated, 125 mL separatory funnel through which hot boiling water was added at the same rate as it was distilled. The initial volume of water added to the 1000 mL flask was 500 mL and heated with a heating mantle. The whole system was insulated with glass wool and aluminum foil to about 2.5 cm below the distilling head elbow (to verify that no bumping or foaming took place); a 30 cm, water cooled, West condensor was attached to the claisen adapter at that point. The distillate was collected in a round bottom flask cooled by a water-ice bath. Preliminary steam distillations of tarbush leaves indicated that approximately 350 mL of distillate were necessary to exhaustively distill the oil. The oil was then obtained by steam distillation of 13.7-20.0 g of whole leaves (500 mL of distillate collected), followed by extraction into hexane, drying of the organic phase over anhydrous $MgSO_4$, and evaporation under reduced pressure. A clear yellow oil was obtained in a yield of 0.28-0.34% of fresh leaf weight (0.84-1.01% of dry matter).

Analyses were performed by gas chromatography coupled to mass spectrometry, using a Finnigan ion trap mass spectrometer (EI, 70 eV) in conjunction with a Varian model 3400 gas chromatograph equipped with a DB-5 column (30 m x 0.25 mm fused silica capillary column, film thickness 0.25 μm) using helium as carrier gas (1 mL/min) and a programmed (injector temperature: 220°C, transfer line temperature: 240°C, initial column temperature: 60°C, final column temperature 240°C, 3°C/min) temperature run (18). Identification of oil components was performed by a comparison of their mass spectra with those of authentic compounds or with literature data (18,19), and by a comparison of their relative retention times with those of authentic compounds (by coinjection with standards if necessary), or by comparison of their retention indices with the literature (18,20). The relative amounts (RA) of individual components [average RA for the 5 minute extracts (n=6), steam distilled extracts (n=3) and the composite 5 day extracts] are expressed as percent peak area relative to total peak area.

Results and Discussion

Table I shows the identity, retention index (on DB-5), and the percent composition of the oil constituents of *F. cernua*. Ninety-four compounds were identified in the oil of tarbush, accounting for over 90% of the composition of the oil. Seventeen of the 77 compounds identified in the ethanol extracts of tarbush were not found in the steam distilled oil. However, 13 of these 17 compounds were found only in amounts of 0.1% or less in the extract and only two of them [selin-11-en-4α-ol (1.2%) and flourensiadiol (44.6%)] were present in any substantial amount. Forty compounds identified in the oil were not present in the 5 min ethanol extracts. Five of these forty compounds were, however, found after further extraction (5 days) of the plant material. Furthermore, only 10 of the 40 components were present in greater than 0.1% yield in the oil and of these only three were found in amounts equal to or greater than 1.0% [artemisia triene (1.4%), α-eudesmol (6.9%) and 7-epi-α-eudesmol (1.0%)].

The presence of flourensadiol in the 5 min ethanol extract was the major factor in the difference

between the ethanol extract and the oil. The two chemical profiles were otherwise qualitatively similar (see above). Minor quantitative differences between individual components of the oil and the extract were noticed, but were generally less pronounced when the 5 day extract was taken into account. Myrcene, δ-3-carene, limonene, β-caryophyllene, germacrene-D and β-eudesmol were clearly present in higher proportions (more in accordance with results obtained with the steam distillation) in the second, longer ethanol extraction (Table I) than in the 5 min extraction. These results provide insight into which compounds might be closer to the leaf surface, less tightly bound to the plant matrix, and more likely involved in herbivore-plant interactions.

The absence of flourensadiol from the oil might be explained by its hydrophylic properties. Flourensadiol was not found in the residue or the distillate of the steam distillation of tarbush leaves even after salting out of the aqueous phases and extraction with diethyl ether. In a separate experiment we found that flourensadiol was extracted from the leaf surface by water, was extracted from the aqueous extract by diethyl ether after salting out with NaCl, and was not extracted from the leaf surface by hexane even after 20 min of shaking. These results indicate that flourensadiol may be too hydrophylic to be steam distilled and undergoes decomposition under these conditions.

Other compounds with different profiles for ethanol extracts and the oil were two monoterpene alcohols (yomogi alcohol and artemisia alcohol), and the monoterpene artemisia triene (all of which possess the same irregular carbon backbone). In the ethanol extract, artemisia alcohol (5.5%) was the main monoterpene compound, whereas yomogi alcohol (0.3%) was present only in very small proportions and artemisia triene was absent. On the other hand, the oil contained a much reduced proportion of artemisia alcohol (2.3%), a large increased proportion of yomogi alcohol (2.7%), and a definite presence of artemisia triene (1.4%). The observed difference might be due to the distillation extracting components from the leaves which the ethanol did not. On the other hand, the difference might be due to the conditions of the steam distillation altering the composition of the oil by either distilling certain components preferentially over others or by rearrangement or decomposition of the original components. In order to distinguish between these possibilities a steam distillation of the ethanol extract of the leaves was performed. Neither hexane nor diethyl ether extracts of the salted out aqueous residue of the distillation contained any of these compounds, indicating no component was distilled preferentially over another. A hexane extract of the distillate showed the presence of (and, a subsequent ether extraction of the salted out residue, the absence of) artemisia alcohol, yomogi alcohol and artemisia triene, in a 36:128:1 ratio respectively, indicating that the steam distillation was indeed responsible for rearrangements of compounds present in tarbush leaves. Studies conducted by Poulter et al. (21) indicated that artemisia alcohol was stable under mild hydrolysis conditions (65°C, 12 h, 50% dioxane/water, three fold molar excess 2,6-lutidine, 1 equivalent dinitrobenzoic acid), although its dinitrobenzoate derivative was found to readily undergo hydrolysis into a mixture of yomogi alcohol and artemisia alcohol (87% and 13%, respectively), with artemisia triene also produced in the case of methanolysis. Whether yomogi alcohol and artemisia triene were generated during steam distillation from artemisia alcohol or from some other precursor found in the leaves of *F. cernua* is not yet clear and is the target of current research at this laboratory.

In summary, the oil and ethanol extracts of tarbush were found to consist of a complex mixture of compounds. The differences between the two isolation methods can be attributed to a combination of factors including the solubility and lability properties of some of the components under the conditions used. Most of the oil and extract components were found in only small amounts with few components being prominent in either profile. Differences in the oil and the ethanol extract suggested that the ethanol extracts were more representative of the components with which a potential herbivore might at first interact and were therefore well suited for use in the examination of response variables in herbivory studies.

Table I. Constituents of the oil and ethanolic extract of *Flourensia cernua*

Compound	RI	Oil[a]	EtOH extract[a,1]
(E)-2-hexenal	855	< 0.1	-
santolina triene	908	-	< 0.1
tricyclene	926	< 0.1	t
artemisia triene	928	1.4	-
α-thujene	931	-	< 0.1 (0.1)
α-pinene	938	0.5	0.5 (1.8)
camphene	954	0.2	1.3
thuja-2,4(10)-diene	958	< 0.1	-
sabinene	976	< 0.1	0.2 (1.2)
β-pinene	980	0.2	0.3 (0.4)
6-methyl-5-hepten-2-one	985	< 0.1	-
myrcene	991	3.8	0.4 (5.5)
yomogi alcohol	997	2.7	0.3
α-phellandrene	1004	0.1	-
δ-3-carene	1011	3.0	0.3 (5.2)
α-terpinene	1018	0.3	t
p-cymene	1025	< 0.1	< 0.1
limonene	1031	6.6	0.7 (12.4)
1,8-cineole	1033	0.4	0.4
(Z)-β-ocimene	1039	< 0.1	t
phenylacetaldehyde	1043	< 0.1	-
(E)-β-ocimene	1050	0.5	< 0.1 (0.2)
γ-terpinene	1061	0.4	t
artemisia ketone	1061	-	t
cis-sabinene hydrate	1068	-	0.3
cis-linalool oxide (furanoid)	1074	< 0.1	-
artemisia alcohol	1083	2.3	5.5 (0.2)
p-mentha-2,4(8)-diene	1087	< 0.1	-
terpinolene	1089	0.1	-
trans-sabinene hydrate	1096	-	< 0.1
linalool	1099	< 0.1	-
p-mentha-1,3,8-triene	1111	< 0.1	-
trans-pinocarveol	1139	< 0.1	< 0.1
camphor	1144	-	t
neoallo-ocimene	1145	0.2	-
trans-verbenol	1146	-	t
isoborneol	1158	< 0.1	t
cis-chrysanthemol	1163	0.9	0.4
borneol	1166	3.3	2.0 (< 0.1)
terpinen-4-ol	1177	0.9	t
m-cymen-8-ol	1180	< 0.1	-
p-cymen-8-ol	1183	< 0.1	-
(Z)-3-hexenyl butyrate	1186	< 0.1	-
α-terpineol	1189	0.1	t
myrtenol	1194	< 0.1	-
trans-carveol	1217	t	-
cuminaldehyde	1240	< 0.1	-
carvone	1242	< 0.1	-
δ-elemene	1339	< 0.1	-(0.1)
α-cubebene	1352	< 0.1	t
neryl acetate	1365	< 0.1	-
cyclosativene	1368	< 0.1	t (t)
α-ylangene	1373	< 0.1	t
α-copaene	1376	0.1	< 0.1 (0.2)
β-bourbonene	1385	0.1	0.1
β-cubebene	1391	0.1	< 0.1 (0.5)
cis-jasmone	1394	0.5	t

Table I. Continued

Compound	RI	Oil[a]	EtOH extract[a,1]
α-cedrene	1411	< 0.1	-(0.4)
β-caryophyllene	1419	1.9	0.3 (13.8)
trans-α-bergamotene	1435	< 0.1	- (< 0.1)
α-guaiene or aromadendrene	1440	< 0.1	-
α-humulene	1454	0.9	< 0.1 (4.0)
seychellene	1461	0.1	< 0.1
α-acoradiene	1464	< 0.1	- (t)
drima-7,9(11)-diene	1469	< 0.1	< 0.1
β-cadinene	1474	0.2	-
γ-muurolene	1476	0.3	0.1
γ-curcumene	1479	0.4	t
germacrene D	1480	2.3	0.4 (18.3)
β-selinene	1485	0.3	0.3 (1.1)
cis-β-guaiene	1490	0.1	-
viridiflorene	1493	0.6	-
epi-cubebol	1494	-	0.1
bicyclogermacrene	1495	t	t (0.4)
α-muurolene	1498	0.2	t
δ-selinene[2]	1506	1.6	0.2 (0.7)
β-curcumene	1510	< 0.1	-
γ-cadinene	1513	0.4	0.2
cis-calamenene	1521	-	< 0.1
δ-cadinene	1523	1.4	t (0.3)
cadina-1,4-diene	1532	< 0.1	t
α-cadinene	1538	0.1	-
α-calacorene	1542	< 0.1	-
elemol	1548	0.7	t (0.3)
germacrene B	1557	0.2	t
(E)-nerolidol	1562	< 0.1	-
caryophyllene alcohol or ledol	1568	0.7	0.6
germacrene D-4-ol	1575	-	< 0.1
spathulenol	1576	0.5	0.1
caryophyllene oxide	1582	0.3	0.4
viridiflorol	1590	2.6	2.7
β-oplopenone	1607	0.1	t
10-epi-γ-eudesmol	1619	0.2	-
1-epi-cubenol	1628	-	t
γ-eudesmol	1631	4.6	0.2
hinesol	1639	1.1	- (0.1)
epi-α-muurolol	1642	0.7	t
cubenol	1642	0.7	t
himachalol	1646	0.4	-
β-eudesmol	1650	24.5	0.6 (10.2)
selin-11-en-4α-ol	1653	-	1.2
α-eudesmol	1653	6.9	-
7-epi-α-eudesmol	1658	1.0	-
bulnesol	1668	-	t
methyl-epi-jasmonate	1675	-	< 0.1
α-bisabolol	1682	0.5	-
8-cedren-13-ol	1686	-	0.3
oplopanone	1735	-	< 0.1
xanthorrizol	1749	< 0.1	-
nootkatone	1798	0.1	< 0.1
flourensiadiol	1867	-	44.6

[1] Values in parenthesis are for corresponding values found in the 5 day ethanol extracts; [2] tentative identification (by M.S. only); [a] average % of relative area (peak area relative to total peak area); RI= retention index as determined on DB-5 using the homologous series of n-hydrocarbons; - = not found; t = trace (<0.01%)

Acknowledgment

The authors would like to thank Dr. David G. I. Kingston for providing an authentic sample of flourensadiol.

References

1. F. P. Mathews, *The Toxicity of the Ripe Fruit of Blackbrush or Tarbush (Flourensia cernua) for Sheep and Goats.* Texas Agricultural Experiment Station, Bulletin No. 664 (1944).
2. J. W. Dollahite and T. J. Allen, *The toxicity of the fruit of Flourensia cernua (Tarbush) (Blackbrush).* Southwestern Vet., **28**, 113-117 (1975).
3. M. O. Dillon, *A systematic study of Flourensia (Asteraceae, Heliatheae).* Fieldiana Bot., **16**, 1-66 (1984).
4. R. E. Estell, E. L. Fredrickson, D. M. Anderson, W. F. Mueller and M. D. Remmenga, *Relationship of tarbush leaf surface secondary chemistry to livestock herbivory.* J. Range Manage., **47**, 424-428 (1994).
5. E. L. Fredrickson, J. Thilsted, R. E. Estell and K. M. Havstad, *Effects of chronic ingestion of tarbush (Flourensia cernua) on ewe lambs.* Vet. Human Toxicol., **36**, 409-415 (1994).
6. R. E. Estell, E. L. Fredrickson and K. M. Havstad, *Secondary chemistry of the leaf Surface of Flourensia cernua.* Biochem. Sys. Ecol., **22**, 73-77 (1994).
7. M. Aregullin-Gallardo, *Chemical and biological significance of benzofurans and benzopyrans in the Asteraceae.* Dissertation, University of California, Irvine (1985).
8. F. Bohlmann and M. Grenz, *Über Inhaltsstoffe der Gattung Flourensia.* Chem. Ber., **110**, 295-300 (1977).
9. M. V. Naidu and G.S.K. Rao, *Synthetic studies in aromatic hemiterpenes of natural origin: Part VI - Synthesis of 7-methoxy-2,2-dimethyl-6-vinylchromene (Anhydroencecalinol), a natural product isolated from Flourensia cernua.* Indian J. Chem., **19**, 313-315 (1980).
10. D.G.I. Kingston, M. M. Rao, T. D. Spittler, R. C. Pettersen and D. L. Cullen, *Sesquiterpenes from Flourensia cernua.* Phytochemistry, **14**, 2033-2037 (1975).
11. D.G.I. Kingston, M. M. Rao and T. D. Spittler, *Isolation and structure determination of flourensic acid, A new sesquiterpene of the eremophilane type.* Tetrahedron Letters, **20**, 1613-1616 (1971).
12. R. C. Pettersen, D. L. Cullen, T. D. Spittler and D.G.I. Kingston, *The crystal and molecular structure of flourensiadiol, a natural product sesquiterpene isolated from a West Texas shrub.* Acta Cryst., **B31**, 1124-1127 (1975).
13. M. M. Rao, D.G.I. Kingston and T. D. Spittler, *Flavonoids from Flourensia cernua.* Phytochemistry, **9**, 227-228 (1970).
14. M. O. Dillon, T. J. Mabry, E. Benson, M. L. Bouillant and J. Chopin, *New flavonoids from Flourensia cernua.* Phytochemistry, **15**, 1085-1086 (1976).
15. T. D. Spittler, *Organic constituents of Flourensia cernua.* Dissertation, State Univ. New York, Albany, Diss. Abstr. Int. B. , **35**(2), 742 (1974).
16. X. A. Dominguez, M. E. Gómez, A. Gómez, A. N. Villarreal and C. Rombold, *Physical data on the essential oils of five Compositae plants.* Planta Med., **19**, 52-54 (1971).
17. M. P. Doyle and W. S. Mungall, *Experimental Organic Chemistry.* John Wiley & Sons, New York (1980).
18. R. P. Adams, *Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy.* Allured Publ. Corp., Carol Stream, Illinois USA (1995).
19. Finnigan Standard Format NIST MS Library, National Institute of Science and Technology, Maryland (1990).
20. G. Buchbauer, B. Remberg, L. Jirovetz and A. Nikiforov, *Comparative headspace analysis of living and fresh cut Lime tree flowers (Tiliae flores).* Flav. Fragr. J., **10**, 221-224 (1995).
21. C. D. Poulter, L. L. Marsh, J. M. Hughes, J. C. Argyle, D.M. Satterwhite, R. J. Goddfellow and S. G. Moesinger, *Model studies of the biosynthesis of non-head-to-tail terpenes. Rearrangements of the chrysanthemyl system.* J. Amer. Chem. Soc., **99**, 3816-3823 (1977).