

The Volatile Oil Composition of Fresh and Air-Dried Buds of *Cannabis sativa*

Sumir A. Rose* and Mahmoud A. ElSohly**

Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, University, Mississippi 38677, and Department of Pharmaceutics, School of Pharmacy, University of Mississippi, University, Mississippi 38677

Received January 12, 1995

The composition of the steam-distilled volatile oil of fresh and air-dried, indoor-grown marijuana was studied by GC/FID and GC/MS. In all, 68 components were detected of which 57 were fully identified. Drying of the plant material had no effect on the qualitative composition of the oil and did not affect the ability of individuals familiar with marijuana smell to recognize the odor.

The chemical composition of the volatile oil of *Cannabis sativa* L. was the subject of several investigations.¹⁻¹¹ A total of 58 monoterpenes and 33 sesquiterpenes was reported to have been identified from different cannabis preparations in a 1990 review article published by Turner *et al.*¹² In a more recent study, Brenneisen and ElSohly¹³ reported on the GC/MS analysis of cannabis extracts and the use of the entire chromatographic characteristics (not just the cannabinoids) for identification of the country of origin of confiscated marijuana. It was shown that the terpenes play an important part in that classification. Because all the work was carried out on dried material, we decided to compare the chemical composition of the cannabis volatile oil prepared from fresh vs. dried plants and to determine the effect of drying on the individuals' ability to recognize the smell.

Although marijuana odor has long been accepted to be recognizable by dogs, the recognition of the smell by law enforcement officers has recently been challenged in courts by the defense in criminal cases. The purpose of this paper is to identify the major components of the essential oil of cannabis and to study the effect of drying and storage of the plant material on the stability of the characteristic components of the oil odor (or odor of the plant material). Olfactory testing of the different oils by human subjects (familiar with marijuana smell) was also used to verify the characteristic odor.

The volatile oil of fresh and dried marijuana was prepared by steam distillation, and the oil was collected using lighter-than-water volatile oil apparatus. Samples of fresh marijuana buds (15 g each) were acquired, with one sample being extracted immediately. Three samples were allowed to dry and were stored at room temperature in paper bags for 1-week, 1-month, and 3-month periods of time prior to the preparation of the volatile oil. In all cases, the volume of oil was determined and the percentage (*w/w*) was referenced to the fresh weight of the plant material (Table 1). Analysis of the oils was carried out by GC/FID and GC/MS under conditions that allowed direct comparison with reported data on reference terpenes.¹⁴ Both retention times and mass spectral fragmentation were used in the identification of the individual components of the oil. Table 2 shows a list

Table 1. Effect of Drying on the Yield and the Composition of the Volatile Oil of Cannabis

	volatile oil samples ^a			
	A	B	C	D
Percentage of Volatile Oil in Buds (<i>w/w</i>)				
correlated to fresh wt.	0.20	0.22	0.19	0.18
correlated to dry wt.		0.65	0.63	0.57
Percentage of Loss in the Volatile Oil content during drying				
		31.0%	41.5%	55.2%
Composition				
monoterpenes	99.48	86.52	67.01	62.02
sesquiterpenes	0.51	13.64	28.53	35.93
others	0.01	0.82	4.47	2.16

^a A: Volatile oil prepared from freshly collected buds. B: Volatile oil prepared from buds that had been dried at room temperature for 1 week. C: Volatile oil prepared from buds that had been dried at room temperature for 1 week and then stored in a brown paper bag for 1 month at room temperature. D: Volatile oil prepared from buds that had been dried at room temperature for 1 week and then stored in a brown paper bag for 3 months at room temperature.

of all components and their retention times (in seconds) and their percentage in each oil. In addition, for all unidentified components the most significant mass spectral fragments are provided.

The percentage composition is determined based on relative response in the FID chromatograms. The identification of each component is based on direct comparison with authentic samples and/or by comparing the retention time and mass spectral fragmentation with the data published by Adams.¹⁴ The mass spectral library fit (NIST Terpene Library) for all identified components was more than 90%. It should be mentioned that the GC column used in this study was identical to that reported by Adams,¹⁴ and therefore all retention times reported were within three seconds of the reference values.

The data presented in Table 2 show that the fresh bud oil is composed mainly of monoterpenes (92%), with 7% sesquiterpenes and approximately 1% other chemical classes such as simple ketones and esters. The two principal monoterpene components are β -myrcene and limonene, representing ca. 67% and 16% of the oil, respectively.

Of the 68 components detected in the chromatogram of the fresh bud oil, 57 were identified (see Table 2). These include 2 hydrocarbon ketones, 1 aliphatic alcohol ester, 23 monoterpenes, and 31 sesquiterpenes. Three of the identified monoterpenes and 14 sesquiterpenes

* Research Institute of Pharmaceutical Sciences.

** Department of Pharmaceutics.

¹ Abstract published in Advance ACS Abstracts, December 1, 1995.

Table 2. Changes' Composition of the Volatile Oil of Cannabis Prepared from Fresh and Dried Buds

number	component	area ^a	percentage			
			A ^b	B ^c	C ^d	D ^e
1	heptanone, 2-methyl-4-	230	0.27	0.30	0.26	0.16
2	heptanone, 3-methyl-4-	801	0.16	0.20	0.14	0.11
3	α -thujene (ariganene)	207	0.15	0.28	†	0.13
4	α -pinene (pinene, β -)	319	1.11	1.12	1.19	1.05
5	camphene	549	0.53	0.49	0.21	0.40
6	cadinene (thujene, α (13)-)	379	†	†	†	NTD
7	β -pinene (tricyclic, nonpinene, pseudopinene, β -myrcene (12-1000))	396	2.23	2.44	2.31	2.32
8	β -myrcene (12-1000)	464	67.11	53.72	38.74	33.53
9	hexyl acetate	419	0.23	0.39	1.93	0.14
10	α -terpinene (terpinene)	458	†	0.63	†	†
11	limonene (limonene, mesol, acyclopitene)	451	18.38	17.20	17.66	16.26
12	1,8-cineole	455	†	†	†	†
13	cis-camphor	459	0.04	†	†	0.15
14	γ -terpinene	515	0.08	0.03	†	0.10
15	tercanone	602	0.24	0.28	0.30	0.20
16	terpinolene (isoterpinolene)	609	0.82	0.79	1.19	0.18
17	linalool (β -linalool, bisylal alcohol)	632	2.80	2.51	3.58	5.97
18	β -fenchol (fenchol, α , norbornanol, β -sabinene hydrate (cis-chujanol))	673	0.35	0.41	0.77	1.0
19	isodienol	683	0.21	0.25	0.37	0.34
20	bornol (camphol)	713	0.07	†	†	0.09
21	bornol (camphol)	763	0.09	0.13	0.19	0.38
22	terpinol-4-ol	820	0.06	†	†	†
23	α -terpineol	852	0.21	0.28	0.37	0.54
24	cis-carveol	952	†	†	†	NTD
25	bornol acetate (bornyl acetate)	1097	†	†	†	†
26	tricyclic	1322	†	†	†	0.37
27	α -copaene (aglaenene)	1334	†	†	†	†
28	β -elemene	1373	†	†	†	0.19
29	(Z)- α -cis-bergamotene	1454	†	†	†	0.09
30	β -caryophyllene	1442	1.33	2.31	6.14	5.45
31	(Z)- α -bis-bergamotene	1485	0.10	0.11	0.33	0.45
32	α -guaiene	1491	0.28	0.47	0.83	1.18
33	α -cadinene	1518	†	†	†	0.11
34	α -humulene (α -caryophyllene)	1527	0.36	0.64	1.93	2.63
35	cis- β -farnesene (farnesene, (Z)- β -)	1557	3.14	0.27	0.55	0.29
36	α -tricyclic	1546	†	†	†	†
37	γ -curculone	1587	†	†	†	0.09
38	curcuminone	1599	3.07	0.13	0.38	0.21
39	β -selinene (cudicene-4/14,11-diene, viridiflorene (ledene))	1806	3.12	0.20	0.42	0.55
40	viridiflorene (ledene)	1826	†	†	†	†
41	α -selinene (cudicene-3,11-diene)	1831	3.17	0.35	0.67	0.63
42	α -longipinene	1836	†	†	†	0.11
43	trans- α -farnesene	1867	0.58	1.02	2.21	2.71
44	β -bisabolene	1867	†	†	†	0.17
45	γ -cadinene	1876	†	†	†	†
46	cis- γ -bisabolene	1881	0.66	0.10	0.25	0.28
47	unknown, M ⁺ 204 (m/z: 169, 161, 119, 105, 91)	1889	0.12	0.25	0.65	0.79
48	trans- β -farnesene (farnesene, (Δ)- β -)	1701	†	†	0.10	0.13
49	trans- γ -bisabolene, bisabolene, (β)- γ -)	1721	0.68	1.2	3.66	6.69
50	unknown, M ⁺ 204 (m/z: 169, 161, 183, 105, 91)	1730	†	†	†	†
51	unknown, M ⁺ 204 (m/z: 169, 161, 107, 81, 89)	1740	0.55	1.76	4.47	6.28
52	γ -elemene	1777	0.29	0.37	0.29	0.11
53	trans-nerolidol	1796	†	†	†	0.09
54	caryophyllene oxide	1817	†	†	†	0.14
55	guaiol (caryophyllol, guaiac alcohol)	1876	0.30	0.3	1.35	1.63
56	unknown, M ⁺ 204 (m/z: 169, 161, 183, 106, 91)	1821	0.30	0.60	1.51	1.98
57	unknown, M ⁺ 204 (m/z: 169, 161, 119, 81, 89)	1841	†	†	†	†
58	β -cadinene (fenchol, β -fenchol, α -fenchol)	1907	0.10	0.28	0.46	0.41
59	unknown, M ⁺ 204 (m/z: 169, 93, 60)	1858	†	†	†	0.11
60	unknown, M ⁺ 204 (m/z: 169, 161, 91, 43)	1866	†	†	†	0.07
61	unknown, M ⁺ 204 (m/z: 169, 121, 105, 90)	1976	†	†	†	0.13
62	δ -cadinol (β -cadinol)	1893	0.13	0.31	0.72	1.06
63	α -cadinol (α -cadinol)	2030	0.17	0.46	0.96	1.40
64	unknown, M ⁺ 204 (m/z: 169, 107, 93, 79)	2036	0.34	0.48	1.23	1.69
65	δ -bisabolol	2045	†	†	†	†
66	epi- α -bisabolol	2089	0.14	0.1	0.89	1.15
67	unknown, M ⁺ 204 (m/z: 169, 161, 133, 119, 44)	2092	†	†	0.16	0.16
68	unknown, M ⁺ 184 (m/z: 162, 139, 41)	2193	0.05	0.09	0.19	0.36

^a Components are numbered in order according to their *r_f*. ^b A = Volatile oil prepared from freshly collected buds. ^c B = Volatile oil prepared from buds dried at room temperature for 1 week. ^d C = Volatile oil prepared from buds dried at room temperature for 1 week and then stored in a brown paper bag for 1 month at room temperature. ^e D = Volatile oil prepared from buds dried at room temperature for 1 week and then stored in a brown paper bag for 3 months at room temperature. † = trace (below peak-area threshold). NTD = not detected.

have not been reported previously in cannabis odor or volatile oil. These sesquiterpenes are as follows: cis-sabinene hydrate (cis-chujanol), ipediol, cis-carveol,

α -ylangene, β -elemene, (Z)- α -cis-bergamotene, α -guaiene, α -cadinene, γ -curculone, γ -curcuminone, viridiflorene (ledene), α -longipinene, γ -cis-bisabolene, γ -trans-bisab-

olene, β -eudesmol (β -asinenol), α -eudesmol (α -asinenol), and α -*epi*-bisabolol.

Not unexpectedly, drying the plant material resulted in overall reduction in the percentage of the oil recovered. Most of the loss appears to take place after the first week of drying, with the oil content dropping from 0.29% to 0.20%. The oil contents after drying and storage for one and three months were 0.16% and 0.13%, respectively (see Table 1). The percentage composition of monoterpenes and sesquiterpenes in these oils is also presented in Table 1.

It is evident from Tables 1 and 2 that drying the plant material results in a significantly greater loss of monoterpenes than of sesquiterpenes. However, qualitatively speaking, none of the major components of the oil ($\approx 0.1\%$ of the total) completely disappeared in the drying process.

To evaluate the odor of the different oils, technicians experienced with marijuana smell were asked to identify the smell of the individual oils. The subjects were able to recognize the smell of all oils as that of marijuana.

Experimental Section

Plant Material. *Cannabis sativa* L. (Cannabaceae) plants were grown from seeds of a high potency hybrid under artificial lights with a light/dark cycle of 16/8 h. After six weeks the light/dark cycle was changed to 10/14 h to induce flowering. Cuttings were then prepared from female plants and were rooted in a mixture of potting soil and vermiculite (1:1). The stems of the cuttings were first wetted with water, then with Rootone (The Security Products Co. of Del. Inc., Minneapolis, MN) before being inserted in the potting mixture and thoroughly watered.

After 21 days at 16/8 h light/dark, the rooted cuttings were transplanted into 6-in. pots with potting soil. The transplanted cuttings were grown under the same conditions for 9 weeks and then under 12/12-h cycle for 11 weeks prior to harvesting. The plants were watered daily with a solution of 3/4 tsp Miracle Grow (Starna's Miracle-Gro, Port Washington, NY) per gallon of water.

Preparation of the Volatile Oil. Marijuana buds (60 g) were harvested on 9 Nov 1992, and divided into four equal parts:

1. The volatile oil was prepared immediately from the first part (15 g) by steam distillation (0.29% yield, w/w) (sample A).

2. The second part (15 g) was air-dried at room temperature for one week to afford 9.77 g of dried material from which the volatile oil was prepared by steam distillation (0.20% yield based on wet material, w/w) (sample B).

3. The third part (15 g) was air-dried at room temperature for one week then stored in a brown paper bag for one month to afford 3.7 g dried material from which the volatile oil was prepared by steam distillation (0.16% yield based on wet material, w/w) (sample C).

4. The fourth part (15 g) was air-dried at room temperature for one week then stored in a brown paper bag for three months to afford 3.5 g dried material from which the volatile oil was prepared (0.13% yield based on wet material, w/w) (sample D).

Reference Standards. Reference standards of different monoterpenes, sesquiterpenes, and alkanes were

obtained from Aldrich Chemical Co. Inc. (Milwaukee, WI), Fluka Chemical Corp. (New York, NY), Roth Co. Chemische Fabrik (Karlsruhe, Germany), Sigma Chemical Co. (St. Louis, MO), and Varian Associates (Houston, TX). These reference standards include: tricyclene, α -pinene, sabinene, α -phellandrene, 1-decene, limonene, fenchone, α -terpinol, terpinolene, *trans*-verbenol, *cis*- and *trans*-carveol, α -longipinene, α -cedrene, (–)-isoeledene, α -copaene, β -bisabolgillolene, (–)-isolongifolol, α -humulene, valencene, cuparene, myricyl alcohol, citronellyl acetate, neryl acetate, geranyl acetate, camphene, allo-aromadendrene, *n*-eicosane, and *n*-heneicosane. Solutions were prepared in methanol at concentrations of 10 mg/mL. For GC/MS analysis each standard solution was diluted by mixing 0.1 mL of the standard solution with 0.9 mL of methanol.

Volatile Oil Solutions. For GC/MS analysis the volatile oils (samples A–D) were dissolved in methanol at a concentration of 1 mg/mL.

GC/MS Analysis. A Varian 3300 gas chromatograph interfaced to a Finnigan 700 ion trap detector (ITD) was used. The gas chromatograph was equipped with dual capillary injectors and a flame ionization detector. ITD data were recorded using an IBM XI computer with Finnigan ITDS v.4.1 software. The ITD software is also equipped with a terpene library comprising over 500 entries of monoterpenes and sesquiterpenes. FID data were recorded using a Hewlett-Packard 3392 integrator.

For chromatographic separations a 0.25 mm \times 30 m, 0.25- μ film thickness, DB-5 column (J&W Scientific, Inc.) was used for GC/ITD and a similar column was used for GC/FID under the same conditions: injector temperature 220 °C, transfer line 240 °C (detector temperature for FID 240 °C), oven temperature (programmed) 60–240 °C at 3 °C/min, with a total run time of 60 min, carrier gas He at a linear velocity of 32 cm/s. Injections of 1 μ L were carried out in the split mode at a ratio of 1:20.

Acknowledgment. Supported in part by the National Institute on Drug Abuse (NIDA), contract no. N01DA-4-7404.

References and Notes

- 1) Lousberg, R. J. J. Ch., *Sciencark*, C. A. *Pharm. Weekl.* 1973, 205, 1.
- 2) Hendricks, E.; Majumdar, D. M.; Balazs, S.; Fox, R. *Pharm. Weekl.* 1978, 215, 413.
- 3) Hest, G. V.; St. Hans, M. R.; Bandy, G. R. *Nature* 1978, 245, 432.
- 4) Marjic, L.; Smith, D. M.; Farnilo, C. G. *Nature* 1981, 291, 574.
- 5) Nagan, M. C.; Hanks, K. L.; Nagan, I. C.; Lee, L. *Can. J. Chem.* 1985, 63, 2872.
- 6) Bercht, C. A. L.; Kuppert, F. J. E. M.; Lousberg, R. J. J. Ch.; Sclerisch, C. A.; Svendsen, A.; Karlson, J. U. *N. Scand. Soc. Agr. Ser. F* 1971, 97, 804-98 F.8-231.
- 7) Ebel, E.; Kande, S. *Terpenoids*, 1979, 20, 28-1.
- 8) Bercht, C. A. L.; Pann, M. R. *Dial. Tech.* 1974, 35.
- 9) Schwab, L. *J. Chromatogr.* 1974, 96, 169.
- 10) Hendricks, E.; Mielig, T. M.; Ballmann, E.; Fox, R. *Phytochemistry* 1975, 14, 314.
- 11) Lomazovskis, E.; Vrezer-Parr, G. *J. Chromatogr.* 1985, 219, 120.
- 12) Turner, C. E.; Elschly, M. A.; Booren, E. G. *J. Nat. Prod.* 1980, 43, 109.
- 13) Branninger, G.; Elschly, M. A. *J. Pharmacol. Sci.* 1980, 69, 1645.
- 14) Adams, R. P. *Identification of Essential Oils by Gas Trap Mass Spectroscopy*, Academic Press, New York, 1989.