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HEADSPACE VOLATILES OF MARIHUANA AND HASHISH: GAS CHROMATOGRAPHIC ANALYSIS OF SAMPLES OF DIFFERENT GEOGRAPHIC ORIGIN

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SUMMARY

The sweet odor of marihuana is of interest for instrumental monitoring of illicit drug traffic and for applications in forensic work. Headspace volatiles of marihuana and hashish of different origin are examined by gas chromatography, and relative compositions of 24 samples are compared. No correlation between volatile make up and geography was found, but the profiling procedures are shown to be of use in the forensic problem of relating samples to a common source.

INTRODUCTION

Interest in the volatile aroma constituents of marihuana has developed because of the possibility of monitoring, by instrumental means, illicit traffic in the drug[1]. Information concerning the volatile composition is also useful in forensic work to relate different seizures to a common source in conspiracy cases[2]. Gas chromatography and gas chromatography–mass spectrometry (GC–MS) are ideally suited to the investigation of plant volatiles. These techniques were used in the initial characterization of marihuana by direct sampling of headspace vapors[3]. Seventeen compounds were identified in a standard sample of marihuana obtained from the National Institute of Mental Health (NIMH) and grown from Mexican seed at the University of Mississippi. These results suggested that a study of the variation in volatile composition of different marihuana and hashish samples would be of value in assessing the potential of marihuana vapor analysis for application in drug monitoring and in forensic work. While the cannabinoid composition of marihuana and hashish of different origin have been extensively investigated[4-12], the aroma constituents have received little attention. Studies of the essential oil of marihuana by Nigam *et al.*[13], Strömberg[14], and Bercht *et al.*[15] were limited to a very few samples. Results of the headspace analysis of marihuana and hashish of several different geographic origins are reported in this paper.

EXPERIMENTAL

Apparatus

A Hewlett-Packard 7610A gas chromatograph equipped with flame ionization detectors and interfaced with a Perkin-Elmer PEP-1 gas chromatography data system was utilized. In order to enhance separation of the headspace constituents, each sample was analyzed on two columns of different polarity. Both glass columns were 6 ft. × ¹/₄ in. O.D. × 2 mm I.D. and were operated as follows: Column I, 3% OV-101 on 100–120 mesh Gas-Chrom Q held at 35° for 10 min and programmed at 6°/min to 100° and held (run time 30 min); Column II, 20% Reoplex 400 on 80–100 mesh Chromosorb W AW, held at 35° for 5 min and programmed at 6°/min to 100° and held (run time 30 min). Injector temperature was 200°, detector temperature was 240° and helium was used as the carrier gas at 35 ml/min. Electrometer sensitivity was $1 \cdot 10^{-10}$ A full scale.

Reacti-vials or reacti-flasks (Pierce, Rockford, Ill., U.S.A.) equipped with on–off valves and septa were used as sample vessels. Headspace sampling and injection were accomplished using gas-tight syringes (Precision Sampling, Baton Rouge, La., U.S.A.).

Samples

Samples of marihuana were obtained through the NIMH from the University of Mississippi. The marihuana types were grown under uniform environmental conditions from documented seed stock of different geographic origin and were harvested at maturity. The histories of the various samples are recorded in Table I. The samples consisted of uniformly manicured leaf material obtained from male plants except samples 7 and 8 which were mixtures from male and female plants. Additional rep-

TABLE I

CHARACTERISTICS OF MARIHUANA SAMPLES OF DOCUMENTED GEOGRAPHIC ORIGIN

Sample No.	Seed origin	Generation of plant*	Age of plant at harvest (weeks)	Δ^9-Tetrahydrocannabinol content (%)**
1	Mexico	3rd	18	1.90
2	Mexico	4th	18	1.56
3	Lebanon	2nd	20	0.89
4	Iran	2nd	20	0.86
5	Afghanistan	1st	20	1.44
6	Afghanistan	1st	20	1.23
7	Poland	1st	17	0.17
8	Poland	1st	17	0.30
9	Czechoslovakia	1st	12	0.15
10	Czechoslovakia	1st	12	0.11
11	India	2nd	20	0.98
12	India	1st	20	0.99
13	India	3rd	20	1.23
14	Pakistan	1st	20	1.34

* Grown at University of Mississippi Garden.
** Courtesy of NINH and University of Mississippi.

resentative samples of marihuana of suspected Mexican origin, and samples of hashish of Mideastern origin were obtained from U.S. Customs seizures. The seized marihuana samples varied considerably in composition including leaf, stem, and seed parts, and in age ranging from fresh to dry and/or moldy.

The hashish samples were in powder form. One large seizure of marihuana in excess of twenty tons was sampled to examine the variation in vapor profiles within a large batch of plant material. This seizure, packaged in 60-lb. bales, was highly compressed. After receipt all samples were stored at −25° until used.

Procedure

Marihuana or hashish volatiles were sampled directly from a reacti-vial or flask. Typically 1 g of plant material was equilibrated in the container for 1 h at 65°. A 5-ml volume of headspace vapors was then withdrawn using a syringe and injected into the gas chromatograph. GC data were acquired by the PEP-1 data system and area normalization calculations performed to obtain the relative composition of the headspace. Data from separate determinations on the non-polar OV-101 and polar Reoplex 400 columns were assimilated in determination of the final composition.

RESULTS AND DISCUSSION

Typical headspace chromatograms obtained using the two columns are shown in Fig. 1. Retention time data for the constituents has been previously reported[3]. Based on ascending order of boiling points, three fractions are distinguished: Fraction I consists of low-molecular-weight oxygenated compounds, Fraction II consists of monoterpene hydrocarbons and oxygenated compounds; Fraction III consists of sesquiterpene hydrocarbons. Previous examination of Fraction I components[3] indicated that these are principally acetone, methanol and ethanol and are of little value in characterizing marihuana due to their widespread distribution in plant products. Therefore, area normalizations were performed only over Fractions II and III for components eluting between 5 and 30 min. Percentage composition of the headspace volatiles computed as described above are shown for the documented geographic samples listed in Table II and for seizure samples listed in Table III. In these comparisons response factors were not utilized as our interest was principally in making relative comparisons. The effect of heating on the samples was examined and no statistically significant difference was found in the headspace compositions at room temperature (20°–28°) and after 1 h at 65°. Sampling at the elevated temperature facilitated quantitation of minor components as approximately a 10-fold enhancement in concentration prevailed under these conditions. The reproducibility of the headspace analysis procedure was determined by analyzing the vapors of five samples of a very homogeneous marihuana. The average standard deviation summed for these replicas over all peaks in the chromatogram (Fraction II and III) was taken as a measure of reproducibility, and was used to develop comparisons between different samples as shown in Table IV.

The variation in headspace composition shown in Table II is significant but there appears to be no clear "geographic" trend. The relative abundance of certain constituents varied sufficiently to allow qualitative distinctions to be made between samples. For example, myrcene ranged from 2.9% to 22.6% and limonene 1.7%

TABLE II

COMPOSITION OF HEADSPACE VOLATILES OF GEOGRAPHIC SAMPLES OF MARIHUANA

t = Trace constituent; — = not detected.

Component	Composition (%)[*] of marihuana samples (see Table I for origin)													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Unknown[**]	1.2	1.0	0.9	1.1	1.1	1.1	2.0	2.8	0.3	0.5	t	t	0.5	0.9
α-Pinene	55.0	54.3	54.8	44.6	53.0	47.7	69.2	71.0	58.4	68.9	61.1	67.5	52.2	53.6
Camphene	0.9	1.1	1.0	1.1	1.3	1.5	1.5	1.6	1.1	1.0	0.8	0.9	1.2	1.5
β-Pinene	16.8	13.3	18.1	11.4	12.5	11.8	12.6	12.4	9.8	11.5	11.2	15.4	12.8	13.5
Myrcene	8.3	10.3	11.6	20.9	9.8	11.0	4.0	2.9	22.6	5.9	7.2	2.9	9.2	9.2
Δ³-Carene	0.6	0.2	0.1	0.1	0.7	0.4	0.1	0.2	t	0.2	3.2	0.4	1.6	0.2
α-Terpinene	t	0.3	t	t	t	t	t	t	—	—	—	t	—	t
Limonene	5.4	5.4	10.0	11.4	16.7	18.1	1.7	1.9	2.2	2.5	5.6	5.2	11.6	14.8
β-Phellandrene	t	—	0.2	2.2	t	1.3	t	0.3	0.3	—	1.4	0.4	1.5	2.0
cis-Ocimene	1.2	1.4	0.4	t	0.5	0.1	0.3	0.3	0.2	0.5	0.4	0.8	0.3	0.6
trans-Ocimene	3.2	3.1	0.8	0.9	0.9	0.4	0.4	0.6	0.2	0.7	0.8	2.0	0.8	0.3
γ-Terpinene	t	—	—	0.6	t	0.6	0.3	0.3	0.2	t	0.3	t	0.4	0.4
Terpinolene	0.8	1.6	0.2	1.3	0.5	0.7	1.0	0.7	0.2	t	0.3	0.4	0.6	0.4
2-Methyl-2-hepten-6-one	0.4	0.3	0.3	0.2	0.2	0.6	0.2	0.1	t	0.2	0.6	0.3	0.5	0.2
β-Caryophyllene	3.4	4.2	0.9	1.9	1.8	2.9	2.8	1.8	1.9	3.5	2.5	1.9	2.8	1.1
α-Bergamotene	0.7	0.8	0.1	0.2	0.4	0.3	0.3	0.5	0.3	0.8	0.4	0.2	0.4	0.1
Humulene	0.7	1.1	0.3	0.6	0.5	0.8	1.1	0.6	0.6	0.9	0.7	0.6	0.7	0.1
β-Farnesene	0.8	0.7	t	0.3	t	0.2	0.1	0.2	t	—	0.3	0.2	0.2	t

[*] Based on fraction II and III only.
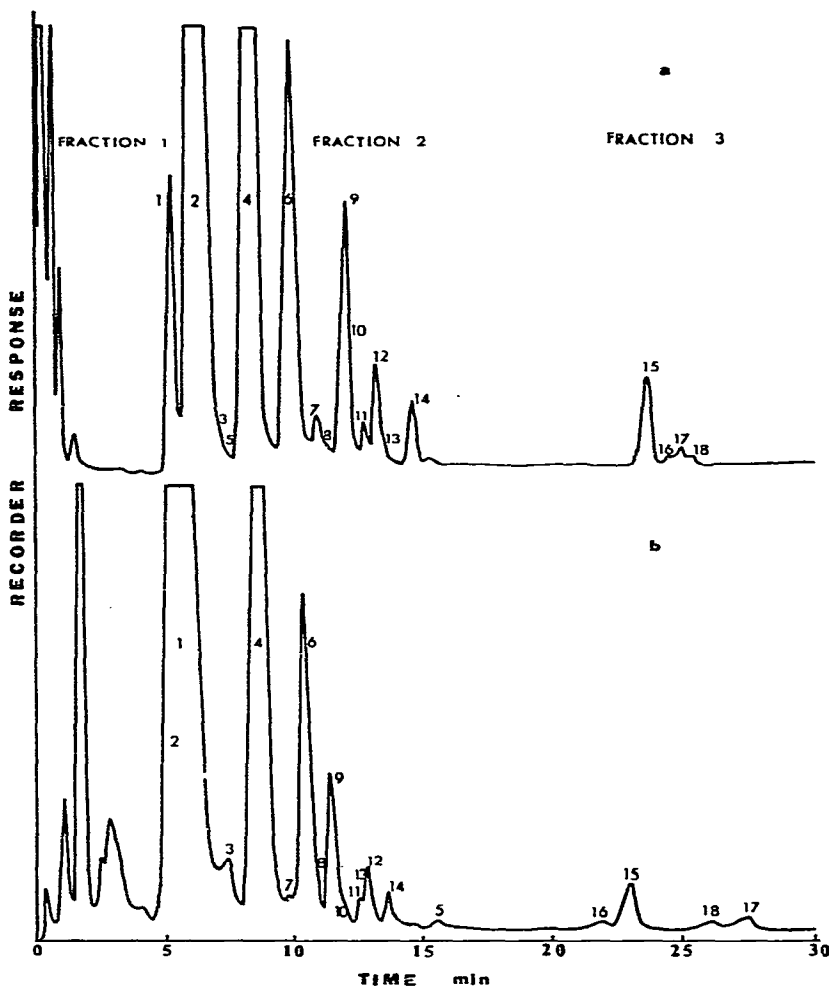[**] Tentatively identified as two compounds: thujene and 1,4-dimethylenecyclohexane.

Fig. 1. Chromatograms of headspace of Mexican marihuana. a, OV-101 column; b, Reoplex 400 column. For GC conditions see text. Peaks: 1 = unknown (tentatively identified as two compounds: thujene and 1,4-dimethylenecyclohexane); 2 = α-pinene; 3 = camphene; 4 = β-pinene; 5 = methyl-2-hepten-6-one; 6 = myrcene; 7 = Δ³-carene; 8 = α-terpinene; 9 = limonene; 10 = β-phellandrene; 11 = cis-ocimene; 12 = trans-ocimene; 13 = γ-terpinene; 14 = terpinolene; 15 = β-caryophyllene; 16 = α-bergamotene; 17 = humulene; 18 = farnesene.

to 18.1%. There is also considerable variation in the composition of the seizure samples of marihuana which are presumably Mexican in origin (Table III). Many of the differences, including failure to detect trace constituents, can be explained on the basis of depletion of volatiles prior to seizure. The relative abundance of β-caryophyllene in the drier samples is understandable; this sesquiterpene constitutes approximately 50% of the essential oil of marihuana, but in fresh samples it makes only a small contribution to the total headspace composition relative to the more volatile monoterpenes. In order to examine the effect of drying on marihuana, a fresh sample was heated at 65° for one month with periodic sampling and analysis. These results con-

TABLE III

COMPOSITION OF HEADSPACE VOLATILES OF SEIZURE SAMPLES OF MARIHUANA AND HASHISH

t = Trace constituent; − = not detected.

Component	Composition (%)* of marihuana samples							Composition (%)* of hashish samples		
	1	2	3	4	5	6	7**	8	9	10
Unknown***	1.4	2.3	0.3	1.6	1.5	1.5	3.0	6.4	4.5	13.6
α-Pinene	46.2	65.5	48.0	57.8	60.2	55.9	52.7	38.5	36.1	35.5
Camphene	1.7	1.8	1.0	0.7	t	t	1.1	1.7	15.6	0.7
β-Pinene	16.4	18.2	16.0	17.6	15.6	15.5	7.4	16.0	17.3	16.2
Myrcene	9.5	5.4	5.6	5.7	1.6	2.5	3.1	19.9	1.0	16.4
Δ³-Carene	0.9	0.7	t	t	t	−	0.6	0.3	−	t
α-Terpinene	t	t	−	t	−	t	t	−	−	−
Limonene	9.1	2.4	3.3	2.9	1.9	2.3	0.9	11.3	9.1	9.1
β-Phellandrene	−	t	−	t	−	−	0.2	1.2	0.5	1.0
cis-Ocimene	1.1	0.3	0.3	1.2	0.6	1.1	0.4	0.3	−	0.3
trans-Ocimene	3.2	0.5	0.4	2.0	1.1	0.8	1.6	0.4	−	0.2
γ-Terpinene	0.2	0.3	−	t	−	−	t	0.2	−	0.2
Terpinolene	3.6	0.4	0.3	1.2	0.9	1.6	0.7	0.8	0.5	0.3
2 Methyl-2-hepten-6-one	0.1	t	0.6	0.3	−	−	0.4	0.1	0.6	0.3
β-Caryophyllene	3.5	1.3	15.4	5.7	9.5	14.8	11.6	1.5	5.1	5.3
α-Bergamotene	1.1	0.3	4.7	1.1	2.0	6.8	7.6	0.1	0.7	0.9
Humulene	0.8	0.3	3.1	1.1	3.3	2.3	3.8	0.3	2.1	1.4
β-Farnesene	0.3	0.1	0.8	0.7	0.6	1.0	2.1	0.2	2.2	0.7

* Based on Fraction II and III only.
** Sampled from 20-ton seizure.
*** Tentatively identified as two compounds: thujene and 1,4-dimethylenecyclohexane.

TABLE IV

STATISTICAL COMPARISON OF VAPOR PROFILES OF VARIOUS CANNABIS SAMPLES

Average σ/peak *

5 Samples of a very homogeneous batch**	2 Samples from within one bale of 20-ton seizure (non-homogeneous)	5 Samples from different bales of 20-ton seizure (non-homogeneous)	Marihuana seizure sample (Table III) 1 and 2	Hashish seizure sample (Table III) 8 and 9
0.43	0.35	1.19	2.06	2.53

* Computed by summing the standard deviations between samples for each peak.
** Standard Mexican marihuana (Sample 1, Table II).

firmed the presence of a higher proportion of sesquiterpene constituents as the sample dried; β-caryophyllene increased 3.3-fold. In addition, unexpected increases were noted in the relative concentration of camphene (3.7-fold) and limonene (2.3-fold), which may have resulted from thermally induced rearrangement of certain monoterpenes. Otherwise, the profiles remained quite similar over the month long sampling period.

The hashish samples gave vapor profiles which were quite similar to those of marihuana. One difference was the appearance in greater proportion of a peak eluting prior to α-pinene on the OV-101 column which has not yet been positively identified. In separate GC–MS experiments using a 30-m glass capillary column, analysis of hashish essential oil has shown this peak to be composed of two compounds tentatively identified as thujene and 1,4-dimethylenecyclohexane.

The data in Table IV show the reproducibility of the headspace sampling technique and demonstrate its utility in characterizing cannabis samples. The similarity in profiles found for different bales in the 20-ton seizure sample is somewhat surprising considering the gross heterogeneity of this material. Profile differences of statistical significance were clearly apparent in the comparisons of seizure samples 1 and 2, and 8 and 9. While such results may not be considered definitive in evaluating cannabis products for same source, it was found that the data are especially valuable when used in conjunction with other results such as cannabinoid analyses.

CONCLUSIONS

Based on the variations found in this study determination of origin by volatile composition alone does not seem promising. Results for the seizure samples of Mexican marihuana point out the variation possible within a given origin and may reflect to a significant extent the prior treatment of the sample. On the other hand, the variation in composition of the geographic samples grown under uniform environmental conditions and subjected to the same treatment suggests that genetic factors may operate in developing differences in volatile make-up. This combination of variables makes origin determination from volatile profiles of seizure samples very difficult. As seen from Table IV, headspace profiles are more useful in the forensic problem of relating two seizures to the same source or in developing useful information about the history of the sample. The procedure is rapid and, with careful sampling, gives re-

producible results. Also of interest is the facile detection of volatile adulterants often added in the illict traffic to camouflage the aroma of marihuana. For example, *p*-di-chlorobenzene has been detected in seizure samples using this method.

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