ESSENTIAL OILS AND THEIR CONSTITUENTS

XXIX.[1] THE ESSENTIAL OIL OF MARIHUANA: COMPOSITION OF GENUINE INDIAN CANNABIS SATIVA L.

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ABSTRACT

The essential oil obtained by hydrodistillation of freshly harvested Indian *Cannabis sativa* L. was found to contain the following constituents that have not previously been reported: α-pinene, camphene, β-pinene, α-terpinene, β-phellandrene, γ-terpinene, linalool, *trans*-linalool oxide, sabinene hydrate, α-bergamotene, terpinene-4-ol, β-farnesene, α-terpineol, α-selinene, curcumene, and caryophyllene oxide. The presence of trace amounts of two alcohols and of an α,β-unsaturated ketone, for which gas chromatographic and spectral characteristics are recorded, was also detected.

INTRODUCTION

Marihuana, *Cannabis sativa* Linn., is, like opium, an important drug-bearing plant that grows wild and is also cultivated in many parts of the world. In 1896 Wood, Spivey, and Easterfield (1) examined volatile fractions obtained from plants grown in India, and reported the isolation of a terpene ($C_{10}H_{16}$, b.p. 170–175 °C) and a sesquiterpene ($C_{15}H_{24}$, b.p. 258–259 °C). About half a century later Simonsen and Todd (2) studied the essential oil obtained from Egyptian hashish. They found that its lower boiling fraction consisted mainly of *p*-cymene in admixture with small amounts of 1-methyl-4-isopropenyl-benzene and an unidentified optically active material. The higher boiling fraction of the oil was found to be largely composed of humulene. These authors failed to isolate any myrcene, a hydrocarbon that they assumed Wood *et al.* (1) had obtained, suggesting that it was polymerized in their product "prepared years ago from hashish of uncertain age". Dutt (3) subsequently established the presence in Indian *C. sativa* L. of *p*-cymene, myrcene, dipentene (limonene), and caryophyllene, along with unidentified sesquiterpenes and sesquiterpene alcohols. He reported also the presence of a compound designated as *p*-cymedene (b.p. 179–180 °C; 2-bromodibromide, m.p. 187–189 °C). Martin, Smith, and Farmilo (4) have recently confirmed that some of these are constituents of the essential oil distilled from plants that grow wild in Ontario and Quebec, Canada.

This paper presents the results of a more detailed investigation of the composition of the essential oil obtained from male plants of *C. sativa* L.

EXPERIMENTAL

Samples

Male and female plants of authentic wild *C. sativa* L. from the province of Jammu-Tawi, Kashmir State, India, were collected separately during their flowering period in the month of May. Two light yellow oils with similar characteristic odours were obtained from them in yields of 0.1% by hydrodistillation. Some physicochemical constants for the oil from male plants were d_{20}^{20} 0.9012, n_D^{20} 1.495, α_D^{20} −11.8°, acid value 1.24; and for that from female plants, d_{20}^{20} 0.9012, n_D^{20} 1.494, α_D^{20} −11.6°, acid value 1.48. The gas chromatograms that were obtained for both products under the same experimental conditions were almost identical. For this reason only the essential oil from male plants was analyzed in detail.

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Fractional Distillation of Crude Isolate

The essential oil obtained by hydrodistillation (60 g) was fractionated under reduced pressure in a Towers column. Five cuts were collected, which are described in Table I.

TABLE I

Fractional distillation of essential oil of *Cannabis sativa* L.

Fraction	Yield (%)	Pressure (mm)	Temp. (°C)	n_D^{20}
1	10.2	23	62–70	1.468
2	6.2	10	82–84	1.484
3	66.0	7	98–104	1.498
4	2.0	7	104–107	1.499
5	10.0	7	107–115	1.501

Chromatography of Fraction 5

On gas chromatographic analysis, fraction 5 appeared to be composed of sesquiterpene hydrocarbons and oxygenated derivatives. It was therefore chromatographed on 120 g of Woelm, neutral grade I alumina, and eluted successively with petroleum ether, benzene, ether, and alcohol. Elution with petroleum ether yielded 3.63 g of product (fraction 5-A) and with benzene led to 1.86 g (fraction 5-B), which were further analyzed. Recoveries from elution with ether and alcohol were negligible.

Examination of Fractions 3, 4, and 5-A

These fractions consisted primarily of sesquiterpene hydrocarbons, and were very similar in composition. They were therefore combined, and a 5 g aliquot of the mixture was subjected to column chromatography over 500 g of Woelm, neutral grade I alumina, with petroleum ether (b.p. 60-80 °C) as eluant. Effluent fractions of 20 ml each were collected, the solvent was evaporated, and the isolation and characterization of the constituents of the products that were thus obtained were accomplished by gas chromatography and physicochemical analyses, respectively.

Gas Chromatographic Techniques

For gas chromatography, the apparatus and general procedures that have been described previously (5) were used.

Identification and Determination of Essential Oil Constituents

Gas chromatographic effluents were identified by the serial dilution technique and by comparison of the infrared spectra of eluates (collected in carbon tetrachloride and purified by re-chromatography under optimal conditions of temperature and flow rate) with those of authentic reference standards available in the authors' collection or with published data (6). Quantitative results were obtained from measurements of peak areas, as previously reported (7, 8).

RESULTS AND DISCUSSION

The complex composition of the essential oil is illustrated in Fig. 1. All materials collected during the course of this study, including crude products, isolates obtained by distillation, and fractions recovered by column chromatography, were subjected to gas chromatographic analysis to determine the composition of the essential oil, which is given in Table II.

α-Pinene, β-pinene, and myrcene.—These monoterpenes were found almost exclusively in fraction 1. Trace amounts only occurred in fraction 2.

Camphene and α-terpinene.—Minute amounts of these hydrocarbons were found in fraction 1.

Limonene, β-phellandrene, γ-terpinene, and p-cymene.—Limonene and β-phellandrene formed the major portion of fraction 1. Small amounts only were found in fraction 2. γ-Terpinene and *p*-cymene were identified as minor constituents of fraction 1, and trace amounts of them were detected in fraction 2. Neither of these fractions contained any 1-methyl-4-*iso*propenylbenzene, a hydrocarbon that Simonsen and Todd (2) reported to

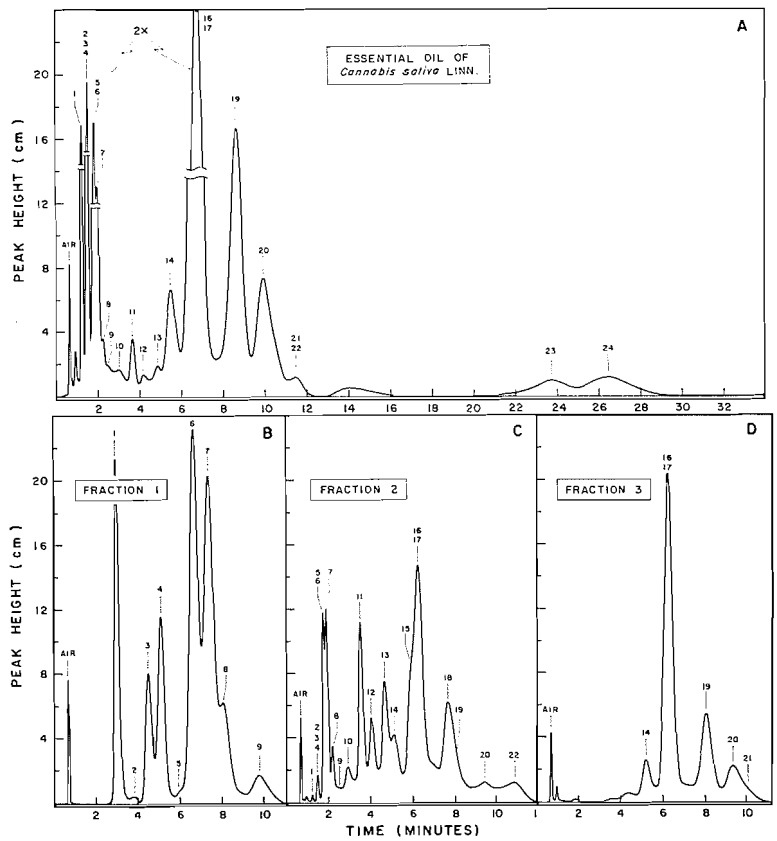

FIG. 1. The result of gas chromatography of oil of *Cannabis sativa* L. Column: Reoplex 400 (20%) on acid-washed chromosorb W. Temperature: A, C, and D,170 °C; B,110 °C. Sample volume: A, 5 λ; B and C, 1.5 λ; D, 1 λ. Helium flow: 75 ml/min.

be a constituent of Egyptian hashish. Its presence in the essential oil was questioned recently by Naves (9).

Linalool and trans-linalool oxide.—These terpenoids were found in fraction 2. *cis*-Linalool oxide, which usually is present with the *trans*-isomer in essential oils, was not detected.

Sabinene hydrate.—This alcohol was identified as a constituent of fraction 2. Its re-chromatography for the purpose of further purification was accompanied by marked decomposition to α- and β-phellandrene and α- and γ-terpinene (10). Hence, some of the β-phellandrene and some α- and γ-terpinene that are reported as constituents of the essential oil (Table II) must be considered as artefacts produced during analysis. Because of the presence of only a small amount of sabinene hydrate (0.4%), no α-phellandrene could be isolated.

Terpinene-4-ol and α-terpineol.—These alcohols were isolated from fraction 2 after repeated gas chromatographic purification.

α-Bergamotene, caryophyllene, β-farnesene, β-humulene, α-selinene, and curcumene.—These sesquiterpenes were identified as components of fraction 3, 4, and 5-A. They also occurred as trace constituents of fraction 2. Caryophyllene and β-farnesene were not resolved by gas

TABLE II

Composition of oil of *Cannabis sativa L.*

Peak No.	Constituent	Relative retention time: Temp., 110 °C, Reference Limonene	Relative retention time: Temp., 170 °C, Reference Caryophyllene	%
1	α-Pinene	0.45	0.18	1.3
2	Camphene	0.56	0.23	0.1
3	β-Pinene	0.67	0.23	0.8
4	Myrcene	0.76	0.23	1.3
5	α-Terpinene*	0.92	0.30	0.1
6	Limonene	1.00	0.30	2.8
7	β-Phellandrene*	1.10	0.32	2.7
8	γ-Terpinene*	1.21	0.35	1.3
9	*p*-Cymene	1.46	0.40	0.4
10	Alcohol A		0.46	0.2
11	Linalool oxide		0.55	0.8
12	Linalool		0.63	0.2
13	Sabinene hydrate		0.74	0.4‡
14	α-Bergamotene		0.81	5.0
15	Terpinene-4-ol		0.93	0.4
16	Caryophyllene		1.00	45.7
17	β-Farnesene		1.00	5.1
18	α-Terpineol		1.22	0.6
19	β-Humulene		1.30	16.0
20	α-Selinene		1.48	8.6
21	Curcumene		1.72	1.4
22	α,β-Unsaturated Ketone		1.72	0.2
23	Alcohol B		3.64	1.6
	Caryophyllene oxide†			1.7
	Unidentified			1.5

*Partial artefact resulting from degradation of sabinene hydrate under experimental conditions.
†Determined by gas chromatography of essential oil using a column of Reoplex 400 (10%) deposited on acid-base washed Chromosorb W (11).
‡Percentage based on the sabinene hydrate that remained unchanged during gas chromatography.

chromatography under the experimental conditions but they were effectively separated by column chromatography, and cyclic sesquiterpene was eluted more rapidly than the aliphatic hydrocarbon. Gas chromatographic analysis of the column chromatographic fractions permitted quantitative determinations.

Caryophyllene Oxide.—The presence of this terpenoid in *C. sativa* L. was deduced from the characterization of peak No. 24 on the gas chromatograph (see Fig. 1) as that of an aldehyde identical with one that is formed by rearrangement of caryophyllene oxide during gas chromatography (11). Its occurrence in the essential oil was established unequivocally by gas chromatographic analysis of fraction 5-B and of a pure reference standard with a column of Reoplex 400 (10%) on acid-base washed Chromosorb W, from which the epoxide was recovered unchanged.

Unidentified compounds.—These included two alcohols (peak No. 10, alcohol A; peak No. 23, alcohol B) and an α,β-unsaturated ketone (peak No. 22). Alcohol A and the α,β-unsaturated ketone occurred in fraction 2, whereas alcohol B was isolated from fraction 5-B. Alcohol A displayed characteristic infrared absorption at 1 280, 1 252, 1 180, 1 127, 1 070, 933, 930, and 840 cm^{-1}; alcohol B exhibited distinct bands at 1 298, 1 242, 1 162, 1 098, 988, and 918 cm^{-1}; and the unsaturated ketone showed marked absorption at 1 677, 1 645, 1 445, 1 428, 1 370, 1 265, 1 236, 1 180, 1 168, 1 137, 1 110, 1 057, 1 045, 1 018, 930, 891, and 840 cm^{-1}.

Composition of the Essential Oil

Data on the quantitative composition of the essential oil of *C. sativa* L. were obtained by correlating the results of fractional distillation with those of column and gas chromatography. They are summarized in Table II.

Value of the Experimental Findings

Previous work by the authors has shown that the geographical and botanical origin of medicinal plants can be determined with a high degree of accuracy by microanalysis of their essential oils (8, 12–16). Studies that will present such data for oil of marihuana will be the subject of a forthcoming publication.

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