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ALKANES OF THE ESSENTIAL OIL OF *CANNABIS SATIVA*

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Key Word Index—*Cannabis sativa*; Cannabinaceae; essential oil; *n*-alkanes; methyl and dimethyl alkanes.

Abstract—A waxy fraction obtained by column chromatography of the essential oil of *Cannabis sativa* consists of *n*-alkanes ranging from C_9 to C_{39}, 2-methyl and 3-methyl alkanes and some dimethyl alkanes. The qualitative and quantitative composition of this fraction has been compared with the alkane fraction obtained by extraction of the herb.

INTRODUCTION

In previous reports concerning the essential oil of *Cannabis sativa* the presence of cannabinoids, the analysis of the mono- and sesquiterpene hydrocarbons and the location of the essential oil together with the cannabinoids in the glandular trichomes have been described [1–3].

In the present work, the essential oil of *Cannabis* was submitted to column chromatography to divide the essential oil into fractions containing the hydrocarbons and the oxygen containing compounds. During this procedure two fractions were obtained which contained a waxy substance, which proved to be a mixture of alkanes after a preliminary GLC investigation. The two fractions were combined and then investigated by means of GC–MS in more detail. The results were compared with those of the alkanes obtained by extraction of *Cannabis* herb from the same origin. Qualitative and quantitative data concerning the alkanes, present as minor components, can possibly furnish information concerning the geographic origin of Cannabis samples [4]. In an earlier report special attention has been paid to the overlapping during GLC of C_{26}–C_{30} *n*-alkanes with the cannabinoids [5].

RESULTS

The results obtained by GLC on OV-1 and OV-17 columns, using reference *n*-alkanes indicated that the alkane fraction of the essential oil consists of *n*-alkanes and other compounds, which could be identified as Me and diMe alkanes by means of GC–MS. The compounds are listed in Table 1 together with their quantitative composition. The MS of 3-Me C_{31} and 2-Me C_{32} showed fragments corresponding with M^+-43 and M^+-29 with a relatively high intensity, indicating that these compounds are possibly accompanied by 2-Me C_{31} and 3-Me C_{32}. GLC also did not show a sharp peak for either compound.

The qualitative and quantitative composition of the alkane fraction obtained by extraction of dry Cannabis herb by means of petrol and purification on a silica gel column is also given in Table 1. The yield of this fraction was about 0.1%. Several compounds of the alkane fraction have not yet been identified, but their GLC R_t suggests that they are also 2- or 3-Me alkanes.

DISCUSSION

For the first time the presence of *n*-alkanes ranging from C_9 to C_{39}, together with several methyl and dimethyl alkanes have been identified in the essential oil of *Cannabis*. Alkanes are usually present in the epicuticular waxes of the leaves, where they have a protective function [6]. A review of the significance of the presence of *n*-alkanes and branched alkanes has already been given [7–9].

It is clear, however, that the quantitative composition of the alkanes obtained from the essential oil does not represent the true situation, because the oil has been obtained by steam distillation and the volatility of the alkanes decreases with their MW. When the quantitative composition of the alkane fraction, obtained by extraction, is compared with that of the alkane fraction obtained by distillation, the major compound *n*-C_{29} is present in 55.8% and 10.65% respectively and that the percentages of the alkanes below *n*-C_{27} in the alkanes obtained by extraction are lower than those of the corresponding alkanes obtained by distillation. The percentage of the alkane fraction in the essential oil was 0.7% and the yield of essential oil was about 0.09%, calculated on dry plant material. The yield of the alkane fraction obtained by extraction was about 0.1%. The conclusion can be drawn that only a small proportion of the total alkanes is removed by distillation.

This paper furnishes us new data concerning the qualitative and quantitative composition of the alkanes present in a certain strain of *Cannabis*. The methods mentioned here may be used to reveal the composition of the alkane fraction of other samples of *Cannabis sativa* L. (or other vegetable material) which is of possible interest for botanical or other (forensic) reasons.

EXPERIMENTAL

Plant material. A botanical description has already been given [3]. Voucher specimens are available at our Laboratory.

Table 1. *n*- and branched alkanes present in the alkane fraction of the essential oil and the alkane fraction obtained by extraction of *Cannabis sativa* leaves

Compound	M^+	Formula	Identification (GC–MS)	Alkane fraction oil essential (%)	alkane-fraction obtained by extraction (%)
1	128	C_9H_{20}	n-C_9H_{20}	*t*	n.d
2	142	$C_{10}H_{22}$	n-$C_{10}H_{22}$	*t*	n.d
3	156	$C_{11}H_{24}$	n-$C_{11}H_{24}$	*t*	*t*
4	170	$C_{12}H_{26}$	n-$C_{12}H_{26}$	1.12*	0.36
5	184	$C_{13}H_{28}$	n-$C_{13}H_{28}$	4.74	0.57
6	212	$C_{15}H_{32}$	3,6-dimethyl $C_{13}H_{26}$	0.64	0.07
7	198	$C_{14}H_{30}$	n-$C_{14}H_{30}$	2.84	0.62
8	226	$C_{16}H_{34}$	2,6-dimethyl $C_{14}H_{28}$	6.80	0.69
9	212	$C_{15}H_{32}$	n-$C_{15}H_{32}$	0.97	0.74
10	226	$C_{16}H_{34}$	n-$C_{16}H_{34}$	1.65	0.36
11	254	$C_{18}H_{38}$	2,6-dimethyl $C_{16}H_{32}$	0.45	0.45
12	240	$C_{17}H_{36}$	n-$C_{17}H_{36}$	0.78	0.17
13	268	$C_{19}H_{40}$	3,6-dimethyl $C_{17}H_{34}$	1.83	0.33
14	268	$C_{19}H_{40}$	3,7-dimethyl $C_{17}H_{34}$	0.19	*t*
15	254	$C_{18}H_{38}$	n-$C_{18}H_{38}$	1.94	0.32
16	282	$C_{20}H_{42}$	3,6-; 3,7-dimethyl $C_{18}H_{36}$†	2.14	0.36
17	268	$C_{19}H_{40}$	n-$C_{19}H_{40}$	1.31	0.22
18	282	$C_{20}H_{42}$	n-$C_{20}H_{42}$	1.20	0.22
19	296	$C_{21}H_{44}$	n-$C_{21}H_{44}$	1.42	0.22
20	310	$C_{22}H_{46}$	3-methyl $C_{21}H_{43}$	0.18	*t*
21	310	$C_{22}H_{46}$	n-$C_{22}H_{46}$	1.38	0.17
22	324	$C_{23}H_{48}$	n-$C_{23}H_{48}$	2.61	0.33
23	338	$C_{24}H_{50}$	3-methyl $C_{23}H_{47}$	0.18	*t*
24	338	$C_{24}H_{50}$	n-$C_{24}H_{50}$	2.77	0.28
25	352	$C_{25}H_{52}$	2-methyl $C_{24}H_{49}$	0.75	0.12
26	352	$C_{25}H_{52}$	n-$C_{25}H_{52}$	6.43	1.65
27	366	$C_{26}H_{54}$	3-methyl $C_{25}H_{51}$	0.97	0.45
28	366	$C_{26}H_{54}$	n-$C_{26}H_{51}$	4.64	0.49
29	380	$C_{27}H_{56}$	2-methyl $C_{26}H_{53}$	1.23	0.05
30	380	$C_{27}H_{56}$	n-$C_{27}H_{56}$	8.79	11.72
31	394	$C_{28}H_{58}$	3-methyl $C_{27}H_{55}$	0.94	0.23
32	394	$C_{28}H_{58}$	n-$C_{28}H_{58}$	4.71	2.08
33	408	$C_{29}H_{60}$	n-$C_{29}H_{60}$	10.65	55.80
34	422	$C_{30}H_{62}$	n-$C_{30}H_{62}$	4.07	3.09
35	436	$C_{31}H_{64}$	n-$C_{31}H_{64}$	3.36	9.94
36	450	$C_{32}H_{66}$	3-methyl $C_{31}H_{63}$	0.45	*t*
37	450	$C_{32}H_{66}$	n-$C_{32}H_{66}$	1.94	0.67
38	464	$C_{33}H_{68}$	2-methyl $C_{32}H_{65}$	0.41	*t*
39	464	$C_{33}H_{68}$	n-$C_{33}H_{68}$	1.31	0.48
40	478	$C_{34}H_{70}$	n-$C_{34}H_{70}$	0.82	0.24
41	492	$C_{35}H_{72}$	n-$C_{35}H_{72}$	0.56	0.09
42	506	$C_{36}H_{74}$	n-$C_{36}H_{74}$	0.30	0.05
43	520	$C_{37}H_{76}$	n-$C_{37}H_{76}$	0.19	*t*
44	534	$C_{38}H_{78}$	n-$C_{38}H_{78}$	*t*	*t*
45	548	$C_{39}H_{80}$	n-$C_{39}H_{80}$	*t*	*t*
			Unidentified	10.30%	6.30%

%: Calculated by the normalization method. *t*: $<0.05\%$. n.d: not detected. *:0.xx does not express the accuracy of the method. †: the two GLC peaks partly overlap.

Distillation of essential oil. Oil was obtained by means of steam distillation of the herb according to a previously described method [10]. Yield 0.09%.

Isolation of alkanes from essential oil. Oil (67 g) was submitted to Si gel column chromatography (1.5 m × 4 cm) using resp. 2 l. petrol (bp < 40°), 1 l. petrol–Et_2O (9:1), 1 l. petrol–Et_2O (4:1) etc and Et_2O. The 1st fraction (*ca* 500 ml) contained 0.2 g of a waxy substance. The 2nd fraction (about 1 5.l.) contained 55.7 g of a mixture of mainly mono- and sesquiterpenes. The following fractions contained the oxygen compounds. The second fraction (44.2 g) was submitted to vacuum distillation to remove monoterpene hydrocarbons (10 g) and 29 g of the residual sesquiterpene hydrocarbon fraction was submitted to column chromatography using Si gel impregnated with 25% $AgNO_3$ using the same eluents as before. The 1st fraction from this column also contained 0.2 g of a waxy product. The 2 waxy fractions were combined after a preliminary GLC analysis. Yield: *ca* 0.7% calculated on the total amount of essential oil.

Isolation of alkanes from herb. Dry Cannabis herb (32 g) was extracted 2 × for 30 sec with 500 ml freshly dist. petrol (bp <40°). Combined extracts were concd to *ca* 2 ml by rotary evaporator and submitted to column chromatography on Si gel, using petrol as eluent. The 1st fraction (*ca* 60 ml) contained 33.7 mg waxy substance (*ca* 0.1 %).

GLC. Alkane fractions were investigated, using *n*-alkanes as reference substances on a packed OV-17 column (2 m × 2 mm, 3% OV-17 on Chromosorb W) temp. programmed 70–300° at 8°/min; flow N_2 30 ml/min and on a micropacked OV-1 column (6 m × 1 mm, 3% OV-1 on Chrompack SA) temp. programmed 80–250° at 4°/min; flow N_2 2 ml/min. Injector and detector temp. 250°. GC–MS was performed on a LKB 2191 computerized system connected via a Becker–Ryhage separator using the micropacked OV-1 column under the conditions mentioned above and on a Varian MAT 112 instrument via a split separator under the following condition : column OV-1 (2 m × 2 mm, 3% OV-1 on Chromosorb W) temp. programmed 70–250° at 8°/min, flow He 2 ml/min. In both cases a scan speed of 3 sec/scan (6–500), an ion source temp. of 250° and an electron energy of 70 eV were used.

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