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Interference of alkanes in the gas chromatographic analysis of cannabis products

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Leaves of young marihuana plants (*Cannabis sativa* L.) were found by gas liquid chromatography to contain appreciable amounts of two long-chain alkanes, n-heptacosane and n-nonacosane. These alkanes, along with other straight chain alkanes ranging from C_{19} to C_{32} could also be detected as minor components in a variety of other marihuana and hashish samples. Depending on the polarity of the g.l.c. column used, the alkanes may have retention times similar to those of the major cannabinoids and thus interfere with qualitative and quantitative analyses of the latter. The findings indicate that g.l.c. alone cannot be used as an accurate and reliable technique for cannabinoid analysis, unless the alkanes are previously removed. However, the alkane composition may be of additional advantage in determining the origin of seized cannabis samples.

Gas-liquid chromatography (g.l.c.) is among the methods of choice for the analysis of marihuana and hashish, both for identification and quantitative evaluation of the cannabinoids. Although many stationary phases have been described, there seems to be a preference for relatively low loaded (1–5%) columns of medium polarity, such as OV 17 (Lerner & Zeffert, 1968; Lerner, 1969; Mechoulam, 1970; Gaoni & Mechoulam, 1971; Vree, Breimer & others, 1971; Fetterman, Keith & others, 1971; Verwey & Witte, 1972) and, to a lesser extent, SE 30 (Farmilo & Davis, 1961; Betts & Holloway, 1967; Aramaki, Tomiyasu & others, 1968; Shoyama, Yamauchi & Nishioka, 1970; De Zeeuw, Malingré & Merkus, 1972).

In a recent investigation on the cannabinoid distribution in young marihuana plants (*Cannabis sativa* L.), using g.l.c. on OV 17, we observed two unexpected peaks in the gas chromatograms, appearing in the same area as the cannabinoids. These unknown components were identified as long chain alkanes. Analysis of a variety of marihuana and hashish samples showed that these alkanes, along with a number of homologues are usually present as minor cannabis constituents. We have therefore studied their behaviour in various chromatographic systems in relation to possible interference with cannabinoid identification and quantitation.

MATERIALS AND METHODS

Young marihuana plants were grown either in the open air or in the greenhouse. The seeds for the plants were of unknown origin. Leaves were harvested after the plants had grown to a height of about 50 cm, without having developed any flowers. The leaves (0·5 g) were crushed, extracted twice with fresh 10 ml portions of chloroform and the combined extracts were filtered and evaporated to dryness in a flash evaporator (Büchi). The residue was weighed and redissolved in a small portion

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of chloroform. Other marihuana samples (0·5 g) were from older plants or obtained from various individual sources; hashish samples (0·1 g) were from police seizures. The extraction was the same as above.

A Becker 409 gas chromatograph, equipped with flame ionization detectors, was used for the analyses. Columns were stainless steel, 4 mm i.d. × 2 m, packed with DMCS-treated Chromosorb G-AW, 80–100 mesh. Stationary phases were OV 17, OV 25, SE 30 or QF 1. Carrier gas was nitrogen. Further g.l.c. conditions are given in Table 1.

Table 1. *Conditions for gas-liquid chromatography.*

Stationary phase	Temperature °C			Inlet pressure kg cm^{-2}	Flow ml min^{-1}
	Column	Injection port	Detector		
5% OV 17	250	300	300	2·5	60
5% OV 25	250	300	300	2·3	60
5% SE 30	240	300	300	2·4	50
3% QF 1	200	275	275	2·5	50

Thin layer chromatography was carried out as described by De Zeeuw & others (1972), using light petroleum–ether (80:20) or benzene, in combination with a trough with 10 ml 25% ammonia at the bottom of the chamber, as solvents. Silica gel G (Merck) was the sorbent. Spots were vizualized with Fast Blue salt B.

Alkanes were isolated by chromatographing the chloroform extracts of the young marihuana leaves on a 1 × 20 cm column of basic alumina, activity grade 1 (Merck) using hexane as solvent. Under these conditions, cannabinoids and cannabinoid acids are retained on the column whereas the alkanes elute according to their molecular weight. Fractions were monitored by g.l.c. Purification of the fractions was done by preparative t.l.c. (De Zeeuw & others, 1972) using hexane as solvent and by preparative g.l.c. on 5% OV 17. The alkanes thus obtained were found to be chromatographically pure for at least 99·5% on the stationary phases of Table 1.

Mass spectra of the alkanes were obtained on a MS 9 apparatus (A.E.I.), direct sample inlet, temperature 160°, electron beam energy 70 eV, acceleration 8 kV.

RESULTS AND DISCUSSION

Fig. 1A shows a gas chromatogram on OV 17 of an extract of a young marihuana plant. The only major cannabinoid in the plant is Δ^9-tetrahydrocannabinol (THC), which constituted about 0·7% of dry weight. Cannabidiol (CBD) and cannabinol (CBN) were present as trace constituents. Two of the other major peaks, designated 3 and 8 have retention times very close to cannabichromene and cannabigerol, respectively. Cannabigerol could not be detected, but the shoulder 4 in peak 3 is caused by cannabichromene. Analysis on SE 30, on which cannabichromene does not coincide with the component of peak 3 clearly showed that in this sample cannabichromene is present in larger quantities than CBD. Thin layer chromatography of the sample and of the trapped g.l.c. fractions readily indicated, however, that the components 3 and 8 were not cannabinoids or other phenols. They did not give a colour reaction with Fast Blue salt B and their migration into the solvent front indicated a non-polar structure.

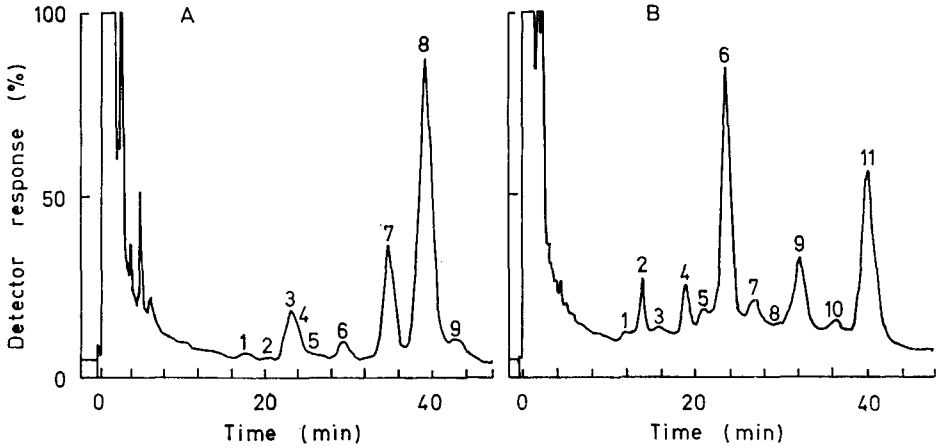

FIG. 1. A Gas chromatogram on OV 17 of an extract of young marihuana leaves. Sample size 5 μl attenuation $\times$ 100, chart speed 0·635 cm min^{-1}. 1 = n-hexaxosane + unknown, 2 = Δ^9-tetra hydrocannabivarol, 3 = n-heptacosane, 4 = cannabichromene, 5 = cannabidiol, 6 = n-octacosane, 7 = Δ^9-tetrahydrocannabinol, 8 = n-nonacosane, 9 = cannabinol.

B. Gas chromatogram on OV 17 of an extract of a hashish sample from India. Sample size 5 μl, attenuation 4 $\times$ 100, chart speed 0·635 cm min^{-1}. 1 = n-pentacosane, 2 = cannabivarol, 3 = n-hexacosane, 4 = Δ^9-tetrahydrocannabivarol, 5 = n-heptacosane, 6 = cannabivarol + cannabidiol, 7 = n-octacosane + trace cannabigerol monomethylether, 8 = Δ^8-tetrahydrocannabinol, 9 = Δ^9-tetrahydrocannabinol, 10 = n-nonacosane + trace cannabigerol, 11 = cannabinol.

Isolation of the two components was then achieved by means of column chromatography on basic alumina, using hexane as solvent. In addition to the components 3 and 8, which were well separated, we encountered a number of minor components in the hexane eluate. The latter could be removed by preparative t.l.c. and g.l.c., thus yielding chromatographically pure fractions of components 3 and 8. High resolution mass spectrometry, coupled with exact mass determinations revealed component 3 to be n-heptacosane (C_{27}) and component 8 to be n-nonacosane (C_{29}). This was confirmed by infrared, nmr, and elementary analysis, Molecular ion peaks were found at *m/e* 380 and *m/e* 408, respectively. The most prominent fragments

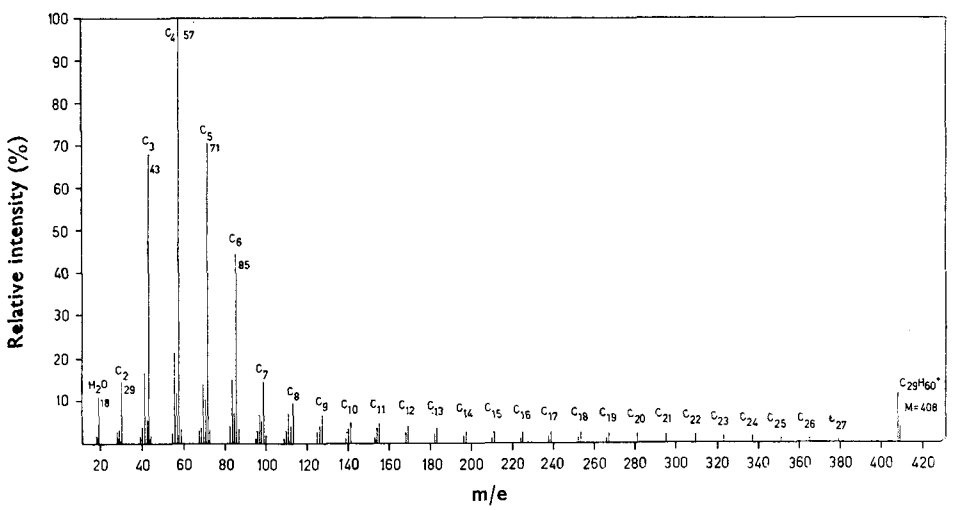

FIG. 2. Mass spectrum of n-nonacosane isolated from young marihuana plants.

in both mass spectra consisted of singly charged alkyl ions of the general formula C_nH_{2n+1}, with base peaks at *m/e* 57 due to butyl ions, $C_4H_9^+$. The mass spectrum of n-nonacosane is given in Fig. 2.

The regular pattern of alkyl ions, showing increasing intensity towards lower mass is characteristic for n-alkanes. Isoalkanes would have a peak of large intensity at the mass corresponding to the formation of an alkyl ion by rupture of the side chain (Ryhage & Stenhagen, 1960; Waldron, Gowers & others, 1961). By the same procedure and with the aid of the mass spectra of n-heptacosane and n-nonacosane, peak 6 could later be identified as n-octacosane (C_{28}). Its mass spectrum was slightly contaminated by impurities due to column bleeding.

The minor components eluting from the column with the major alkane fractions were further analysed by g.l.c. Their small quantities did not allow satisfactory mass spectrometric analysis. However, most of them could be tentatively identified as normal straight chain alkanes, ranging in chain length from C_{19} to C_{32}. This could be done by means of their retention times on four different columns and by the fact that the logarithm of the retention times is a linear function of the carbon number (Louloudes, Chambers & others, 1962; Littlewood, 1970). Authentic alkane standards and the isolated C_{27}, C_{28} and C_{29} alkanes served as references. Two minor components could not yet be identified. They may represent branched alkanes.

The retention times of the alkanes on OV 25, OV 17, QF 1 and SE 30, together with those of the major cannabinoids are listed in Table 2. It is obvious that the alkanes can easily interfere with the qualitative and quantitative analysis of the major cannabinoids. It can also be seen that with more polar columns (SE 30 < QF 1 < OV 17 < OV 25) the retention times of the alkanes shift to smaller values, whereas those of the cannabinoids remain almost the same. Hence, on OV 25 the C_{27}–C_{32}

Table 2. *G.l.c. retention times of major alkanes and cannabinoids in cannabis, relative to CBD.*

Compound	Column			
	5% OV 25	5% OV 17	3% QF 1	5% SE 30
Cannabidivarol	0·62	0·60	0·57	0·59
Δ^9-Tetrahydrocannabivarol	0·85	0·80	0·73	0·76
Cannabivarol	1·08	1·00	1·04	0·89
Cannabichromene	0·95	0·92	0·93	1·02
Cannabidiol	1·00[1]	1·00[2]	1·00[3]	1·00[4]
Δ^9-Tetrahydrocannabinol	1·38	1·35	1·26	1·29
Cannabigerol	1·52	1·52	1·38	1·40
Cannabinol	1·76	1·69	1·81	1·54
n-Alkane C_{19}	s	s	s	0·26
C_{20}	s	s	s	0·32
C_{21}	s	0·17	0·21	0·43
C_{22}	s	0·23	0·28	0·56
C_{23}	0·20	0·30	0·38	0·75
C_{24}	0·27	0·40	0·50	1·00
C_{25}	0·35	0·53	0·66	1·34
C_{26}	0·45	0·70	0·88	1·73
C_{27}	0·57	0·89	1·17	2·29
C_{28}	0·73	1·15	1·54	3·04
C_{29}	0·97	1·54	2·05	3·96
C_{30}	1·24	2·03	2·69	5·17
C_{31}	1·63	2·68	3·55	6·75
C_{32}	2·15	n.r	n.r	n.r

[1] Retention time 22 min 31 s.
[2] Retention time 23 min 48 s.
[3] Retention time 19 min 3 s.
[4] Retention time 20 min 59 s.
s = component in solvent peak.
n.r = not recorded.

alkanes will interfere with a reliable cannabinoid analysis, but on SE 30 interference will be mainly due to C_{20}–C_{26} alkanes.

In order to obtain some information on the occurrence of alkanes in older marihuana plants and in hashish we have screened a variety of samples available in our laboratories and originating from various parts of the world. In all these samples alkanes could be detected, especially the C_{25} –C_{30} alkanes. In most samples alkanes were easily detectable under conditions normally used for cannabinoid analysis. An example is shown in Fig. 1B, representing a gas chromatogram of a hashish sample from India. Samples with a larger proportion of stems usually showed higher quantities of alkanes.

The above findings clearly indicate that gas chromatography alone cannot reliably be used for accurate cannabinoid analyses because of possible interference of alkanes. Although this interference only slightly affects the major peaks of THC, CBD, CBN and their propyl homologues in Fig. 1B, the C_{29} alkane has the same retention time as cannabigerol, while the C_{28} alkane has the same retention time as cannabigerol monomethylether. Moreover, it should be remembered that a change in column operating conditions will, of course, affect the retention times of both alkanes and cannabinoids, but not to the same extent. This may result in other overlappings than those in Fig. 1B. Thus, to get a true picture of the cannabinoid composition in cannabis by g.l.c., alkanes have to be previously removed, preferably by column chromatography over neutral or acidic adsorbents. With basic adsorbents it will be very difficult to elute cannabinoid acids.

The alkanes do not interfere with t.l.c. analysis of the major cannabinoid as the former all migrate into the solvent front and do not react with the Fast Blue salt B spray reagent.

Papers have been published recently by Strömberg (1971, 1972a, b) indicating that the minor constituents of cannabis could be an important factor in determing the geographical origin of cannabis samples and in answering the question whether cannabis samples seized at different places can be assigned to the same source, thus tracing back the lines of distribution. Both questions are of great importance for forensic science and drug abuse control. We feel that a detailed alkane analysis, combined with a reliable cannabinoid analysis, can become a valuable factor in answering these questions. By means of simple column chromatography the alkane fractions can be separated from the cannabinoids as well as from other minor constituents, after which the alkane fractions can be subjected to g.l.c. for rapid qualitative and quantitative analysis.

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