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GAS CHROMATOGRAPHY OF CANNABIS CONSTITUENTS AND THEIR SYNTHETIC DERIVATIVES

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SUMMARY

Twenty-five natural and forty-five synthetic cannabinoids have been identified by gas chromatography–mass spectrometry.

It appeared that the retention times of groups of cannabinoids showed a characteristic pattern. An increase in the side-chain increases the retention time by a fixed amount of 42% per carbon atom. When the position of the side-chain is shifted from the *ortho* to the *para* position of the aromatic ring, the retention time is increased by a factor of 1.3. Reduction of the polarity by methylation and silylation reduces the retention time by a factor of 0.53. Branching of the side-chain results in an increase in the retention time by 12%. Saturation of the double bonds leads to a decrease in the retention time by a factor of 0.80.

INTRODUCTION

The gas chromatography of cannabis extracts has been studied intensively and it has been shown to be a very useful tool for characterising cannabis extracts and the identification of the constituents[1–8]. For instance, it is used in criminology and forensic toxicology[8–10]. On each occasion, evidence should be obtained that the compound with the retention time of, for example, 1,2-tetrahydrocannabinol(1,2-THC*), the major psychotomimetic cannabinoid, is in fact 1,2-THC and that there is no coincidence with other compounds that have the same retention time.

In general, it might be dangerous to identify hashish constituents on the basis of a single retention time only, without further characterisation of the compound. In that event, the use of scarce standard cannabinoids is required, but many of these cannabinoids are not available as reference substances. Further evidence about the structure of a compound can be obtained by derivatisation of the compound, which leads to a second set of retention times. Next to thin-layer chromatography, this method in many instances offers an adequate possibility of identifying the cannabinoids.

The most powerful method involves separation by gas chromatography followed

*Δ1,2-THC (in this paper Δ is omitted) = 1,2-THC according to the monoterpenoid numbering.

by mass spectrometric analysis of the separated compounds (GC–MS). In this way, it was possible to identify unambiguously not only the well known cannabis constituents but also some unknown constituents bearing a propyl and a methyl side-chain[11,12].

From the mass spectra, it could be ascertained that the alicyclic ring system of the cannabinoids dominates the mass spectral fragmentation and that the influence of the other part of the molecule, the aromatic moiety with the side-chain, free OH groups or an ether function, is of minor importance[13,14].

After primary identification of the cannabinoids by mass spectrometry in the combined GC–MS system, we also measured the retention times on an OV-17 column. These retention times showed, for groups of cannabinoids, a characteristic behaviour and in general it can be said that the aromatic moiety now dominates the gas chromatographic behaviour while this moiety was of minor importance in mass spectrometry[14]. It should be clear that when working with the combination of gas chromatography and mass spectrometry, both gas chromatographic and mass spectrometric data are extremely important for the elucidation of cannabis structures. In gas chromatography, the retention time itself is a result of the interaction between a compound and the stationary phase at certain instrumental settings (*e.g.*, temperature, gas flow-rate, column efficiency). This interaction depends on the partition coefficient of the compound between the vapour phase and the liquid phase in the column[15]. Also, relative volatilities are dependent upon activity coefficients and vapour pressures[16]. The relative retention time expresses the ratio of the interactions between the stationary phase and the compounds compared. All of these physicochemical properties are related to the structure of the compounds. Therefore, when one of a series of closely related compounds is assumed to be the standard, the difference in retention time of the other compounds can be related to the difference in structure[17–21]. Many of the cannabis constituents and synthetic derivatives mentioned are not available in sufficient amounts to measure vapour pressures and other physical parameters, so that the interaction of cannabinoids with the stationary phase can only be described in terms of relative retention times in correlation with the structures. Thus, with the aid of reference compounds, changes in structures such as ring closure and the reduction of polarity of cannabis constituents are related to changes in relative retention times.

EXPERIMENTAL

Ether extracts of marihuana and hashish samples were injected into the gas chromatograph and combined gas chromatograph–mass spectrometer. The compounds were identified by means of mass spectrometry[11–14,22,23] and the retention times were measured in centimetres.

It was noticed that there existed a fixed ratio between the retention times of compounds that were structurally related. From this observation, relative retention times were estimated for all the compounds examined. Two or more series of relative retention times obtained on one column could be related to each other when the series had one compound in common that could be considered as the standard. In this way, all of the retention times could be related and 1,2-THC-C5 was considered to be the standard with a *fictive rentention* of 100. The fictive retention of all other compounds examined and identified in the experiments could be calculated, and in this way the fictive retentions given in Table I and the following tables were obtained.

TABLE I

FICTIVE RETENTION OF CANNABIS CONSTITUENTS AND SYNTHETIC DERIVATIVES

Formulae and names are given beneath the table.

Compound[a]	*Type*[b]	*Fictive retention*
Resorcin-O,O-dimethyl	S	0.195
Resorcin-O-methyl	S	0.58
Resorcin	S	0.90
Orcinol	S	1.32
Olivetol-O,O-dimethyl	S	2.82
ortho-1,6-THC-Co-O-methyl	S	4.45
Olivetol-O-methyl	S	4.85
1,6-THC-Co-O-methyl	S	6.10
Olivetol	S	8.10
CBN-Co-O-methyl	S	9.50
CBD-Co	S	10.0
Cannabicyclol-C3[14,33]	N	14.3
1,6-THC-Co	S	15.5
CBN-Co	S	16.3
CBD-C1	N	20.1
1,2-THC-C1	N	23.0
CBD-C5-O,O-dimethyl	S	24.3
ortho-CBD-C5-O,O-dimethyl	S	25.0
Cannabichromene-C3[14]	N	27.0
CBN-C1	N	28.0
CBN-C3-O-TMS	S	28.2
Cannabicyclol-C5	N	30.0
ortho-1,6-THC-C5-O-methyl	S	33.6
Cannabigerol-C3-O-methyl	N	34.8
Molecular weight 300	N	38.0
HHC-C3	S	40.0
HHC-*tert.*-butyl	S	40.5
ortho-1,2-THC-C5-O-methyl	S	40.8
CBD-C3	N	42.5
CBD-C5-O-methyl	S, (N)	44.5
1,2-THC-C5-O-TMS	S	45.0
1,6-THC-C5-O-methyl	S	47.0
1,2-THC-C3	N	50.0
ortho-HHC-C5	S	50.2
1,2-THC-C5-O-methyl	S	54.5
1,6-THC-*tert.*-butyl	S	55.1
CBN-C3	N	60.0
Cannabichromene-C5	N	60.0
CBN-C5-O-TMS	S	62.5
ortho-1,6-THC-C5	S	67.0
Cannabinodiol-C3[18]	N	71.0
CBN-C5-O-methyl	S	74.0
HHC-C5	S	75.0
ortho-1,2-THC-C5	S	76.0
Cannabigerol-C5-O-methyl	N	77.0
Molecular weight 328	N	80.5
CBD-C5	N	81.0
ortho-CBD-C5	N	81.0
1,6-THC-C5	N	84.5
Dihydro-(8,9)-CBD-C5	S	86.0
Tetrahydro-CBD-C5	S	86.5
HHC-C5,α-methyl	S	92.0
1,6-THC-C5,α-methyl	S	95.0
1,2-THC-C5	N	100
Dihydro-(1,2)-CBD-C5	S	105
HHC-C5,α,α-dimethyl	S	106

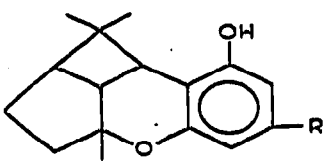
(Continued on p. 212)

TABLE I *(continued)*

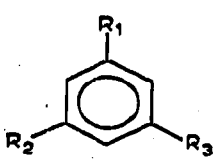
Compound	*Type*	*Fictive retention*
HHC-C6	S	106.5
1,6-THC-C5,α,α-dimethyl	S	114
Cannabigerol-C5	N	117
1,6-THC-C5-α,β-dimethyl	S	124
CBN-C5	N	126
Synhexyl	S	130
Cannabinodiol-C5	N	150
1,6-THC-C5-C7OH	N	
1,6-THC-C5,α-OH	N	
1,6-THC-C5,γ-OH	N	
CBNC5-C7OH	N	
ortho-1,6-di-THC-C5	S	258
1,6-di-THC-C5	S	345

[a] C*n* refers to the number of carbon atoms in the side-chain. HHC is hexahydrocannabinol. α-, β- and γ- refer to the carbon atoms in the side-chain.

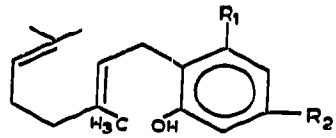
[b] N is a naturally occurring cannabinoid; S is a synthetic cannabinoid.



Substituent	*Compound*
R	
C_3H_7	Cannabicyclol-C3
C_5H_{11}	Cannabicyclol-C5

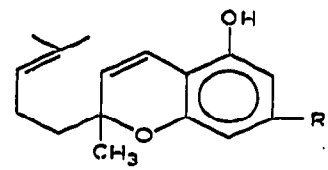


Substituent			*Compound*
R_1	R_2	R_3	
OH	OH	H	Resorcin
O-Me	OH	H	Resorcin-O-methyl
O-Me	O-Me	H	Resorcin-O,O-dimethyl
OH	OH	CH_3	Orcinol
OH	OH	C_3H_7	Divarinol
OH	OH	C_5H_{11}	Olivetol
O-Me	OH	C_5H_{11}	Olivetol-O-methyl
O-Me	O-Me	C_5H_{11}	Olivetol-O,O-dimethyl

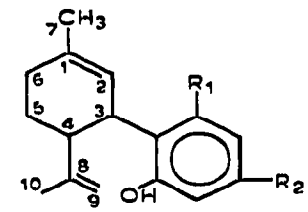


Cannabigerol

Substituent		*Compound*
R_1	R_2	
OH	C_3H_7	Cannabigerol-C3
OH	C_5H_{11}	Cannabigerol-C5
O-Methyl	C_3H_7	Cannabigerol-C3-O-methyl
O-Methyl	C_5H_{11}	Cannabigerol-C5-O-methyl

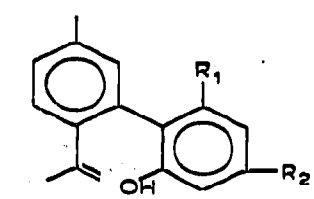


Substituent	*Compound*
R	
C_3H_7	Cannabichromene-C3
C_5H_{11}	Cannabichromene-C5



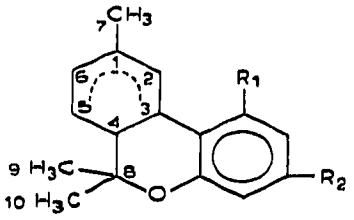
Cannabidiol

Substituent		*Compound*
R_1	R_2	
OH	H	CBD-Co
OH	CH_3	CBD-C1
OH	C_3H_7	CBD-C3
OH	C_5H_{11}	CBD-C5
O-Me	C_5H_{11}	CBD-C5-O-methyl
O-Si$(CH_3)_3$	C_5H_{11}	CBD-C5-O-TMS
C_5H_{11}	OH	*ortho*-CBD-C5



Cannabinodiol

Substituent		*Compound*
R_1	R_2	
OH	C_3H_7	Cannabinodiol-C3
OH	C_5H_{11}	Cannabinodiol-C5



Substituent		*Compound*
R_1	R_2	
OH	H	THC-Co
OH	CH_3	THC-C1
OH	C_3H_7	THC-C3
OH	C_5H_{11}	THC-C5
OH	C_6H_{13}	Synhexyl, double bond 3,4
OH	$C(CH_3)CCCC$	THC-C5,α-methyl
OH	$C(CH_3)(CH_3)CCCC$	THC-C5,α,α-dimethyl
OH	$C(CH_3)C(CH_3)CCC$	THC-C5,α,β-dimethyl
OH	$C(CH_3)(CH_3)C$	THC-C5-*tert.*-butyl
C_5H_{11}	OH	*ortho*-THC-C5
O-Me	C_5H_{11}	THC-C5-O-methyl
O-Si$(CH_3)_3$	C_5H_{11}	THC-C5-O-TMS

Samples

Marihuana samples were obtained from Columbia, Congo, Laos, Indonesia and Brazil.

Hashish samples were obtained from Nepal, Afghanistan, Turkey, Lebanon, Morocco, South Africa and The Netherlands.

Gas chromatography

The gas chromatographs used were H&P 402, H&P 400, Becker 409, H&P 5750 and LKB 9000 instruments, together with a flame ionization detector and an electron bombardment detector (LKB). An LKB 9000 combined gas chromatograph–mass spectrometer was used.

The oven temperature was 180–200°, the separator 240°, the flash heater 230°, the detector 250° and the ion source 290°. The flow-rate of nitrogen was 30 ml/min, hydrogen 30 ml/min, air 150 ml/min and helium 20 ml/min. The recorders were a Moseley 7127A, 1 mV full-scale; a Honeywell, 1 mV; and a Hitachi Perkin-Elmer 165, 1 mV–10 V.

The column was 1.80 m × 3 mm I.D. and the stationary phases were 3% OV-17, 3.8% UCW-98, 3% OV-1, 3% Xe-60 and 3% Apiezon L.

The ionisation potentials used for gas chromatography were 20–40 eV, and for mass spectrometry 20–10 eV[11,12]. The acceleration potential was 3.5 kV and the trap current 60 μA.

Reagents

The methylating reagent was trimethylanilinium hydroxide, and the silylating reagent was trimethylsilylimidazole. Saturation of the double bonds was carried out with H_2/PtO_2 at 1 atm.

RESULTS AND DISCUSSION

Influence of the length of the side-chain on the retention time

The cannabinoids show a very characteristic behaviour on the column in the gas chromatograph. In general, the compounds elute smoothly from stationary phases such as OV-17, OV-1, UCW-98, Apiezon L, Xe-60, etc., all being silicone gum type phases.

When the retention times of the cannabinoids, cannabidiol (CBD), tetrahydrocannabinol (THC) and cannabinol (CBN) with a pentyl (C5), propyl (C3) or methyl (C1) side-chain were compared, it was observed that there existed a fixed ratio between those retention times (Table II and Fig. 1). This ratio was found to be independent of the temperature of the oven, gas flow-rate, apparatus and stationary phase[14]. It appeared experimentally that for separation, a silicone gum type of stationary phase was required and that the chemical structure of the side-chain was the dominant factor

TABLE II

INFLUENCE OF THE LENGTH OF THE SIDE CHAIN ON THE RETENTION TIME

Compound	*Fictive retention*	*Relative retention time*	
CBD-C1[a]	20.1	1.00	
CBD-C3	42.5	2.12	1.00
CBD-C5	81.0	4.04	1.92
1,2-THC-C1	23.0	1.00	
1,2-THC-C3	50.0	2.16	1.00
1,2-THC-C5	100	4.35	2.01
CBN-C1	28.0	1.00	
CBN-C3	60.0	2.15	1.00
CBN-C5	126	4.50	2.10
Cannabinodiol-C3[13]	71.0		1.00
Cannabinodiol-C5[13]	150		2.10
Cannabicyclol-C3[33]	14.3		1.00
Cannabicyclol-C5	30.0		2.10
Cannabichromene-C3[14]	27.0		1.00
Cannabichromene-C5	60.0		2.20
Cannabigerol-C3-O-methyl[27]	34.8		1.00
Cannabigerol-C5-O-methyl	77.0		2.21
Hexahydrocannabinol-C3[27]	40.0		1.00
Hexahydrocannabinol-C5	75.0		1.87

[a] C*n* refers to the number of carbon atoms in the side-chain.

with respect to separation. Of course, the alicyclic ring system itself gives a contribution to the total separation, but this seemed to be of minor importance compared with the side-chain.

From Table II, it can be concluded that when the length of the side-chain is increased by two carbon atoms, the retention time increases by 100%. Thus, $R_t(Cn) : R_t(Cn + 2) = 1:2$. It is possible to calculate the relative retention times of previously "artificial" cannabinoids, which have not been identified as naturally occurring cannabinoids, bearing an ethyl (C2), butyl (C4), hexyl (C6) side-chain, assuming that the retention time increases by 42% per carbon atom.

Let us assume the compound with no side-chain (C0) has a retention time 1.00

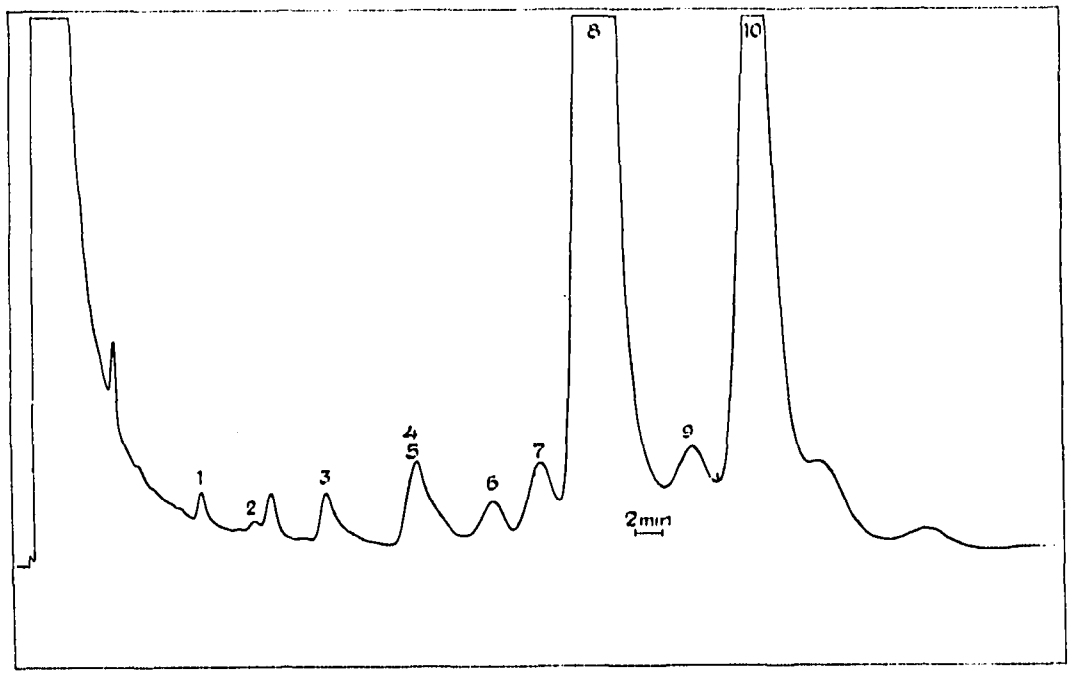


Fig. 1. Gas chromatogram of a sample of marihuana from Brazil, obtained on 3% OV-17 at 200°. 1 = 1,2-THC-C1; 2 = CBN-C1; 3 = 1,2-THC-C3; 4 = CBN-C3; 5 = Cannabichromene-C5; 6 = Cannabigerol-C5-methyl ether; 7 = 1,6-THC-C5; 8 = 1,2-THC-C5; 9 = Cannabigerol-C5; 10 = CBN-C5.

(arbitrary value). Then the relative retention times of the compounds with an increasing side-chain will be:

cannabinoid-Co = 1.00
-C1 = 1.42
-C2 = 2.00
-C3 = 2.84
-C4 = 4.00
-C5 = 5.68
-C6 = 8.00

With 1,6-THC-C5 as standard, the relative retention time of 1,6-THC-Co can be estimated under the given assumptions to be 15.0. This compound was synthesized and the retention time found was 16.1.

Influence of the position of the side-chain in the aromatic ring system on the retention time

In nature, the side-chain is in the *para* position to the ring system, but when the compounds are synthesized, *ortho* substitution also takes place[24–26]. A sample of synthetic 1,2-THC-C5 that was studied, also contained a fraction of the *ortho*-substituted 1,2-THC-C5 and both *ortho*- and *para*-1,6-THC-C5[22] (Figs. 2 and 3). These compounds were identified by GC–MS[22]. The retention times of these four compounds and their O-methylated products show that the position of the side-chain is important for the retention time on the OV-17 column (Table III).

From Table III, it can be derived that a shift of the side-chain from the *ortho* to the *para* position increases the retention time by a factor of about 1.3. Reduction of the polarity of the molecule by methylation does not alter this phenomenon.

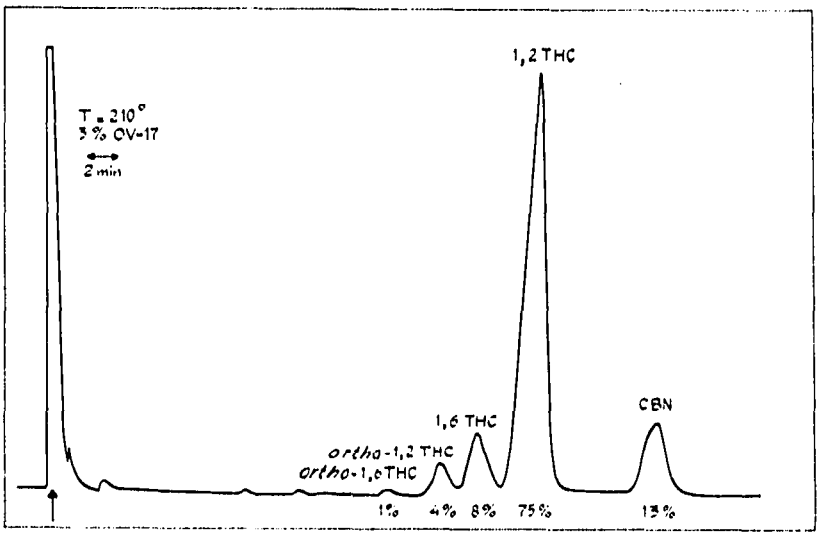
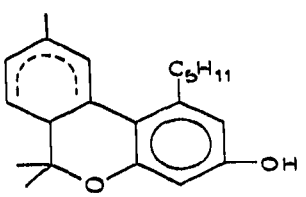
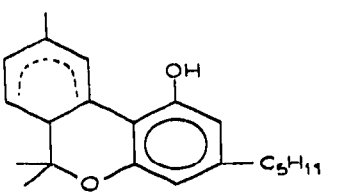


Fig. 2. Gas chromatogram of a sample of synthetic 1,2-THC-C5. Effect of the *ortho–para* substitution of the aliphatic side-chain on the retention time.



(a)

ortho-tetrahydrocannabinol
(—) 1-(*n*-amyl)-3-hydroxy-6,6,9 trimethyl-6*a*,10*a*-*trans*-tetrahydrodibenzo(*b*,*d*) pyran[34]



(b)

para-tetrahydrocannabinol

TABLE III

INFLUENCE OF THE POSITION OF THE SIDE-CHAIN IN THE AROMATIC RING SYSTEM ON THE RETENTION TIME

Compound	*Fictive retention*	*Relative retention time*
ortho-1,2-THC-C5	76.0	1.00
1,2-THC-C5	100	1.31
ortho-1,6-THC-C5	67.0	1.00
1,6-THC-C5	84.5	1.26
ortho-1,2-THC-C5-O-methyl	40.8	1.00
1,2-THC-C5-O-methyl	54.5	1.34
ortho-1,6-THC-C5-O-methyl	33.6	1.00
1,6-THC-C5-O-methyl	47.0	1.40

Influence of reducing the polarity of the aromatic phenol group by methylation and silylation on the retention time

In the interaction between the cannabinoid and the stationary phase, the free OH group may play an important role. The effect of the contribution of the OH group in this interaction can be demonstrated by reducing the polarity by methylation and

silylation. From Table IV it can be derived that reduction of the polarity by methylation and silylation leads to a decrease in the retention time by a factor of 0.53. The influence of the alicyclic ring system is small for those compounds which are closely related in structure. This can be seen for the THC compounds, for example.

TABLE IV

INFLUENCE OF REDUCING THE POLARITY OF THE AROMATIC HYDROXYL GROUP BY METHYLATION AND SILYLATION ON THE RETENTION TIME

Compound	*Fictive retention*	*Relative retention time*	
1,6-THC-C5-O-methyl	47.0	1.00	
1,6-THC-C5	84.5	1.80	
1,2-THC-C5-O-methyl	54.5	1.00	
1,2-THC-C5	100	1.84	
CBD-C5-O,O-dimethyl	24.3	1.00	
CBD-C5-O-methyl	44.5	1.83	1.00
CBD-C5	81.0	3.33	1.83
ortho-1,6-THC-C5-O-methyl	33.6	1.00	
ortho-1,6-THC-C5	67.0	1.98	
ortho-1,2-THC-C5-O-methyl	40.8	1.00	
ortho-1,2-THC-C5	76.0	1.83	
CBN-C5-O-methyl	74.0	1.00	
CBN-C5	126	1.70	
Cannabigerol-C5-O-methyl	77.0	1.00	
Cannabigerol-C5	117	1.52	
1,2-THC-C5-O-TMS	45.0	1.00	
1,2-THC-C5	100	2.22	
CBN-C3-O-TMS	28.2	1.00	
CBN-C3	60.0	2.10	
CBN-C5-O-TMS	62.5	1.00	
CBN-C5	126	2.02	

Influence of branching in the side-chain in 1,6-THC on the retention time

To date, no cannabinoids with branched side-chains have been found in natural hashish and marihuana samples. The influence of branching in the side-chain can be estimated by comparing synthetic analogues of one cannabinoid. In this study, we were able to compare derivatives of synthetic 1,6-THC. From Table V, it can be derived that, as with lengthening of the side-chain, branching leads to an increase in retention time. However, it seems that with branching, steric effects that are not easy

TABLE V

INFLUENCE OF BRANCHING IN THE SIDE-CHAIN ON THE RETENTION TIME

Compound	*Fictive retention*	*Relative retention time*		
1,6-THC-Co	15.5			
1,6-THC-*tert.*-butyl	55.1			
1,6-THC-C5	84.5	1.00		
1,6-THC-C5,α-methyl	95.0	1.12	+0.12	+12%
1,6-THC-C5,α,α-dimethyl	114	1.31	+0.19	+17%
1,6-THC-C5,α,β-dimethyl	124	1.47	+0.16	+12%

to interpret greatly influence the relative increase per carbon atom, which therefore is not simply a constant factor.

Influence of double bonds in cannabinoids on the retention time

All cannabinoids contain double bonds, but at different positions in the molecule. Saturation of these bonds may give an indication of the partial contribution of the double bond to the interaction between cannabinoids and the stationary phase of the column (Fig. 3). The retention time of the compound with the double bond is therefore compared with its saturated analogue, the hexahydrocannabinol, HHC.

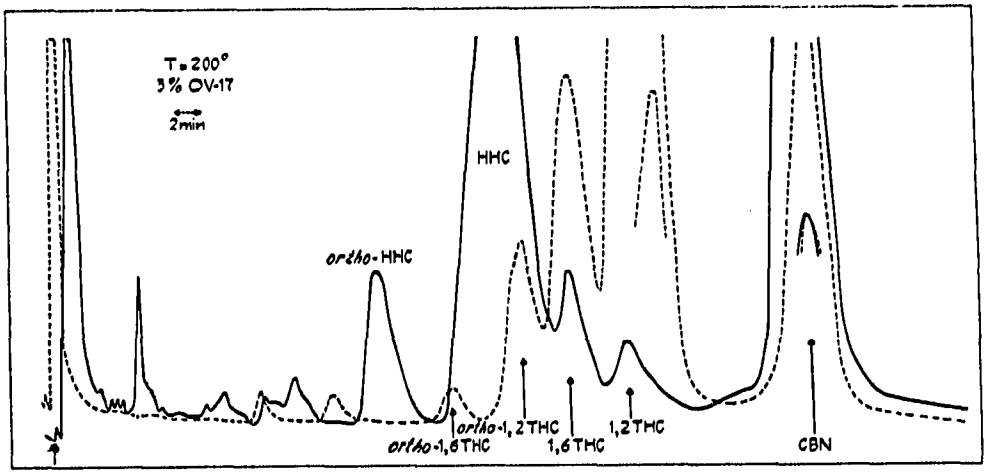


Fig. 3. Effect of saturation on the retention time of the compounds *ortho*- and *para*-1,6- and -1,2-THC. Saturation results in two hexahydrocannabinols, with *ortho*- and *para*-substituted side-chains.

TABLE VI

CONTRIBUTION OF THE 1,2-DOUBLE BOND OF CANNABINOIDS TO THE RETENTION TIME

Compound	*Fictive retention*	*Relative retention time*
HHC-C_3	40.0	1.00
1,2-THC-C_3	50.0	1.25
HHC-C_5	75.0	1.00
1,2-THC-C_5	100	1.33

TABLE VII

CONTRIBUTION OF THE 1,6-DOUBLE BOND IN CANNABINOIDS TO THE RETENTION TIME

Compound	*Fictive retention*	*Relative retention time*
HHC-C_5	75.0	1.00
1,6-THC-C_5	84.5	1.12
HHC-C_5,α-methyl	92.0	1.00
1,6-THC-C_5,α-methyl	95.0	1.03
HHC-C_5,α,α-dimethyl	106	1.00
1,6-THC-C_5,α,α-dimethyl	114	1.07

From Table VI, it can be derived that the retention time is increased by a factor of 1.29 (29%) due to the 1,2-double bond.

From Table VII, it can be derived that the increase in retention time due to the double bond in the 1,6-position is a factor of 1.07 (7%).

The compound synhexyl has a C6 side-chain. The retention time of dihydrosynhexyl or hexahydrocannabinol-C6 must be 42% more than the corresponding HHC-C5. It shifts from 75 to 106.5 (Table VIII).

TABLE VIII

CONTRIBUTION OF THE 3,4-DOUBLE BOND IN CANNABINOIDS TO THE RETENTION TIME

Compound	*Fictive retention*	*Relative retention time*
HHC-C6	106.5	1.00
Synhexyl	130	1.22

TABLE IX

EFFECT OF AROMATISATION OF THE ALICYCLIC RING SYSTEM OF CANNABINOIDS ON THE RETENTION TIME

Compound	*Fictive retention*	*Relative retention time*
HHC-C5	75.0	1.00
CBN-C5	126	1.66
Dihydro(1,2)CBD-C5	105	1.00
Cannabinodiol-C5	150	1.43

From Table IX, it can be derived that aromatisation of the alicyclic ring system of cannabinoids increases the retention time by a factor of 1.54 (54%).

Hence, the partial contribution to the shift of retention time due to the different double bonds is:

1,2-double bond	+0.29	+29%
1,6-double bond	+0.07	+ 7%
3,4-double bond	+0.22	+22%
aromatisation	+0.54	+54%

Let us assume that the double bond in the 5,6-position is chemically identical with that in the 1,6-position in the interaction between the cannabinoid and the stationary phase. In that case, aromatisation may be explained by the addition of the partial contributions of the interactions of the three double bonds:

1,2- (29%) + 3,4- (22%) + 5,6- (7%) = 58%.

Aromatisation of the alicyclic ring system leads to an increase in retention time of 54%, and the increase is 58% when the partial interactions of the double bonds are added. It can therefore be assumed that the aromatisation of hexahydrocannabinol to cannabinol can be represented by the addition of the partial interactions of the individual double bonds.

Influence of the double bonds in cannabidiol on the retention time

For the estimation of the partial contributions of the double bond in cannabidiol

(CBD), the double bonds were reduced with H_2/PtO_2. The compounds obtained were tetrahydrocannabidiol, dihydro-(1,2-)cannabidiol and dihydro-(8,9)-cannabidiol, and were identified by GC–MS[27].

No significant shift in retention time is observed when a double bond is introduced into tetrahydrocannabidiol in the 1,2-position (Table X). With mass fragmentography of the masses 316 and 318, the two compounds in the overlapping peaks in the total ion current recording could be separated.

The introduction of the 8,9-double bond into tetrahydrocannabidiol results in an increase in the retention time shift of +0.22 (22%) (Table XI).

TABLE X

CONTRIBUTION OF THE 1,2-DOUBLE BOND IN CANNABIDIOL TO ITS RETENTION TIME

Compound	*Fictive retention*	*Relative retention time*
Dihydro-(8,9)-CBD-C5	86.0	1.00
Tetrahydro-CBD-C5	86.5	1.00

TABLE XI

CONTRIBUTION OF THE 8,9-DOUBLE BOND IN CANNABIDIOL TO ITS RETENTION TIME

Compound	*Fictive retention*	*Relative retention time*
Tetrahydro-CBD-C5	86.5	1.00
Dihydro-(1,2)-CBD-C5	105	1.22

TABLE XII

CONTRIBUTION OF THE 1,2- AND 8,9-DOUBLE BONDS TOGETHER IN CANNABIDIOL TO ITS RETENTION TIME

Compound	*Fictive retention*	*Relative retention time*
Tetrahydro-CBD-C5	86.5	1.00
CBD-C5	81.0	0.93

The introduction of both the 1,2- and 8,9-double bonds together in tetrahydrocannabidiol results in a decrease in the retention time by −0.07 (−7%) (Table XII).

In general, the introduction of the double bond results in an increase in the retention time. Examples of this behaviour are given with the hexahydrocannabinols. In tetrahydrocannabidiol, the introduction of the 1,2-double bond caused a negative shift in the retention time of −0.5%. This effect cannot be observed accurately, but compared with the increase in the retention times of hexahydrocannabinols when the 1,2-double bond is introduced, the lack of increase in retention time in tetrahydrocannabidiol when the 1,2-double bond is introduced may be considered as a negative shift.

This negative shift is increased with the further introduction of the 8,9-double

bond, which alone gives a positive shift in retention time of 0.22 (22%). Thus the 1,2-double bond introduced into dihydro-(1,2)-CBD results in a negative shift in the retention time of −0.29 (−29%).

Contribution of the alicyclic ring system of cannabinoids to the retention time

All the relationships between the compounds with variations in the aromatic moiety can exist only when the separation between different alicyclic moieties can be performed. If the alicyclic part has no influence, then no separation could be achieved between CBD, THC and CBN. The problem is to establish the relationship between the alicyclic part of the molecule and the interaction with the stationary phase. This problem is as important as are the variations in the structure and interaction of the aromatic moiety. From Table XIII, it can be seen that there is no relationship at first sight between the structure of the compounds and their retention times.

Perhaps an attempt can be made to classify the compounds. Cannabigerol has an aromatic ring with two free OH groups and two long aliphatic chains. In cannabichromene, one of the OH groups is alkylated into a ring.

TABLE XIII

FICTIVE RETENTION OF DIFFERENT CANNABIS CONSTITUENTS

Compound	*Fictive retention*
Cannabicyclol-C5	30.0
Cannabichromene-C5	60.0
Cannabidiol-C5	81.0
1,6-Tetrahydrocannabinol-C5	84.5
1,2-Tetrahydrocannabinol-C5	100
Cannabigerol-C5	117
Cannabinol-C5	126
Cannabinodiol-C5	150

TABLE XIV

RETENTION TIMES OF CANNABICHROMENE AND CANNABIGEROL

Compound	*Fictive retention*	*Relative retention time*
Cannabichromene-C5	60.0	1.00
Cannabigerol-C5	117	1.95

When the retention times of cannabigerol and cannabichromene are compared, the ring closure may be considered as a reduction of the polarity by alkylation (Table XIV).

This idea agrees well with the results of methylation and silylation of the free OH groups, which might indicate that the difference between cannabichromene and cannabigerol is largely explicable on the basis of reduction of the polarity of an aromatic hydroxyl group.

CONCLUSION

For the identification of cannabis constituents, gas chromatography and mass spectrometry are of great importance. With mass spectrometric data, a compound eluting from the gas chromatograph can be identified unambiguously. When only gas chromatographic data are available, as is most often the case, it is possible to obtain more information about the compounds represented in the chromatogram by comparing retention times and ratios to a reference standard. We have tried to show that each part of the cannabinoid molecule makes an individual contribution to the interaction with the stationary phase. Unknown compounds can thus be arranged together on the basis of their gas chromatographic behaviour, and then with a certain amount of probability they can be classified into certain categories, as has been shown in this paper.

For instance, in a sample of Congo marihuana, two compounds are found with molecular weights of 300 and 328. The mass spectrum of the former at 20 eV showed mass fragments of 300 (100%), 258 (95%) and 231 (30%), and the mass spectrum of the latter of 328 (95%), 286 (100%) and 258 (30%). The corresponding masses differ by a mass fragment 28, and thus the compounds may be analogues, bearing a C3 and C5 side-chain. Further information about the origin of the compounds is obtained from the retention times of both compounds. The compound of molecular weight 300 has a fictive retention of 38.0 and the compound of molecular weight 328 has a fictive retention of 80.5. The latter retention time is a factor 2.12 greater than the former, and this factor agrees well with the results of the ratio of retention times of C3 and C5 side-chains. One could use a similar argument for compounds with a C4 and C6 side-chain, but from the biosynthetic point of view this would seem not to be logical.

Hence, from GC–MS data, it can be concluded that the compounds of molecular weights 300 and 328 are analogues with different side-chains.

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