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GAS CHROMATOGRAPHY OF CANNABINOIDS

GAS CHROMATOGRAPHIC BEHAVIOUR OF *cis*- AND *trans*-TETRAHYDROCANNABINOL AND ISOTETRAHYDROCANNABINOL

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SUMMARY

Synthetic side-products of tetrahydrocannabinols (1,6-, 1,2- and iso-THC) were submitted to gas chromatographic–mass spectrometric analysis and the compounds were identified by mass spectrometry. The gas chromatographic behaviour of the THC isomers is very characteristic and there are fixed ratios of the retention times for the *cis–trans*-isomers (1.85), *ortho/para*-isomers (1.20) and iso-THC/1,2-THC (2.67 and 4.80).

INTRODUCTION

In nature, the hydrogen atoms on the 3- and 4-carbon atoms of the main cannabinoids 1,6-, and 1,2-tetrahydrocannabinol (THC) occur in the *trans*-position[1] (monoterpenoid numbering)*. For this reason, the molecule is flat and may act psychotomimetically. Most attention in the synthesis of these two THCs has been paid to the stereochemical synthesis of the *trans*-(3,4)-THCs[2,3]. However, specificity in a particular synthesis does not eliminate side-products (see Fig. 1). This means that in the synthesis of THC and possibly even in nature the *cis*-(3,4)-THCs are present. To anticipate this possibility, gas chromatographic–mass spectrometric (GC–MS) data should be available. FAHRENHOLTZ *et al.*[4,5] and TAYLOR *et al.*[6] synthesized and isolated *cis*-(3,4)-1,2-THC as a side-product in the synthesis of *trans*-(3,4)-1,2-THC and published nuclear magnetic resonance data.

GAONI AND MECHOULAM[1] reported the synthetic side-product iso-THC, in which the phenolic OH group has attacked the C_1 atom to form a pyran ring.

In the literature, few gas chromatographic and mass spectrometric data on

* 1,2-THC = Δ1,2-THC: the Δ is omitted in this paper.

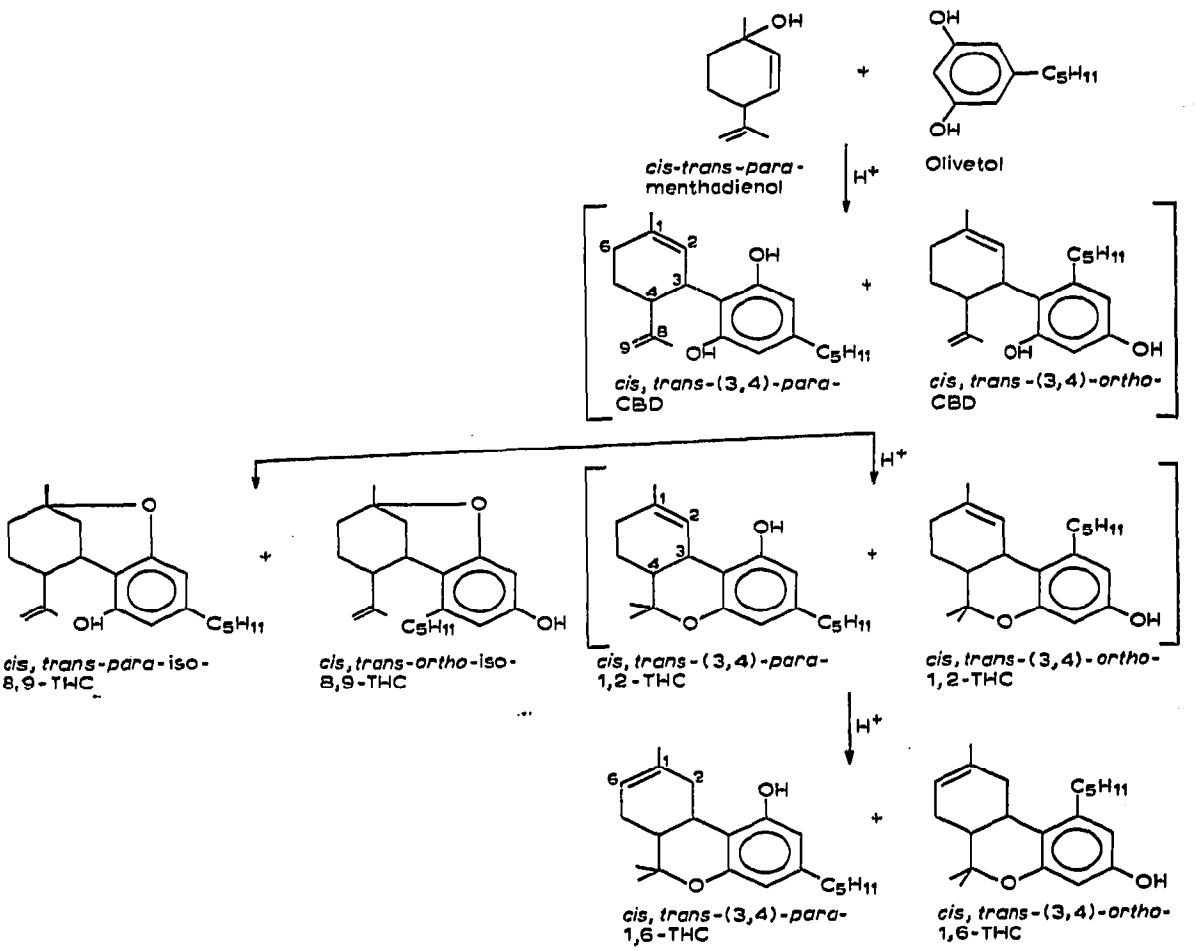


Fig. 1. Synthesis of 1,6-THC according to PETRZILKA and co-workers[2,3].

synthetic cannabinoids have been given. Gas–liquid chromatographic (GLC) analysis of reaction mixtures has rarely been used in order to follow or to modify the reaction. With the aid of the synthetic cannabinoids 1,2-THC, 1,6-THC and 1,6-THC-α-methyl (Table I) and the synthetic side-products formed during their synthesis, we have investigated the influence of the configuration of the hydrogen atoms of the C_3 and C_4 atoms on the gas chromatographic behaviour. With the data obtained, the synthesis of a cannabinoid can be followed qualitatively by means of gas chromatography.

EXPERIMENTAL

Ethereal solutions of synthetic 1,2-THC, 1,6-THC and 1,6-THC-C5-α-methyl were injected into the gas chromatograph and combined gas chromatograph–mass spectrometer. The compounds were identified by means of mass spectrometry, the

TABLE I

FICTIVE RETENTIONS OF SYNTHETIC AND NATURAL CANNABINOIDS

Fictive retention	*Synthetic cannabinoid*[a]	*Fictive retention*	*Natural cannabinoid*[a]
		14	Cannabicyclol-C3
17	*trans*-iso-*ortho*-8,9-THC-C5		
20	*trans*-iso-*para*-8,9-THC-C5	20	CBD-C1
22	*trans*-iso-*ortho*-8,9-THC-C5-α-methyl		
		23	*trans-para*-1,2-THC-C1
27	*trans*-iso-*para*-8,9-THC-C5-α-methyl	27	Cannabichromene-C3
		28	CBN-C1
29	*cis-ortho*-HHC	30	Cannabicyclol-C5
34	*cis-ortho*-1,6-THC-C5-α-methyl	34	Cannabigerol-C3-O-methyl
		38	Molecular weight 300
41	*cis-ortho*-1,6-THC-C5		
41	*cis-para*-HHC		
		42	CBD-C3
46	*cis-ortho*-1,2-THC-C5		
47	*cis-para*-1,6-THC-C5		
		50	*trans-para*-1,2-THC-C3
53	*cis-para*-1,6-THC-C5-α-methyl		
53	*cis-para*-1,2-THC-C5		
		60	CBN-C3
			Cannabichromene-C5
67	*trans-ortho*-1,6-THC-C5		
69	*trans-ortho*-1,6-THC-C5-α-methyl		
		71	Cannabinodiol-C3
77	*trans-para*-HHC	77	Cannabigerol-C5-O-methyl
78	*trans-ortho*-1,2-THC-C5		
		80	Molecular weight 328
		81	CBD-C5
84	*trans-para*-1,6-THC-C5	84	*trans-para*-1,6-THC-C5
95	*trans-para*-1,6-THC-C5-α-methyl		
100	*trans-para*-1,2-THC-C5	100	*trans-para*-1,2-THC-C5
		126	CBN-C5

[a] C1 to C5 refer to the number of carbon atoms in the side-chain[7].

retention times measured in centimetres and the fictive retentions calculated. The fictive retention of *trans-para*-1,2-THC-C5 is arbitrarily set at 100, as described earlier[7]. Fictive retentions and structures are given in Tables I–III and Figs. 2–4.

Gas chromatography

The gas chromatographs used were an H & P 402 and an LKB 9000.

The following conditions were used:

Flame ionisation detector (H & P) and electron bombardment detector (LKB).

Oven temperature 200°; flash heater 230°; detector 250°.

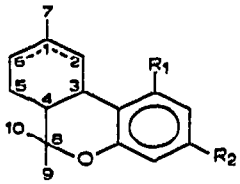
Nitrogen flow-rate 30 ml/min; hydrogen flow-rate 30 ml/min; air flow-rate 150 ml/min.

Recorders: Moseley 7127 A, 1 mV full-scale; Hitachi Perkin-Elmer 165, 1 mV–10 V.

Column in each apparatus: length 1.80 m, 3 mm I.D., packed with 3 % OV-17 on Gas-Chrom Q, 60–80 mesh.

TABLE II

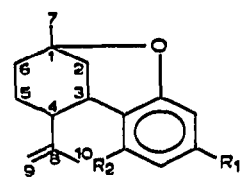
STRUCTURES OF SYNTHETIC CANNABINOIDS



Compound	*Double-bond*	*Position of H atoms at C3 and C4*	R_1	R_2
cis-ortho-1,6-THC-C5	1,6	*cis*	OH	C_5H_{11}
cis-para-1,6-THC-C5	1,6	*cis*	C_5H_{11}	OH
cis-ortho-1,6-THC-C5-α-methyl	1,6	*cis*	$C(CH_3)CCCC$	OH
cis-para-1,6-THC-C5-α-methyl	1,6	*cis*	OH	$C(CH_3)CCCC$
trans-ortho-1,6-THC-C5	1,6	*trans*	C_5H_{11}	OH
trans-para-1,6-THC-C5	1,6	*trans*	OH	C_5H_{11}
trans-ortho-1,6-THC-C5-α-methyl	1,6	*trans*	$C(CH_3)CCCC$	OH
trans-para-1,6-THC-C5-α-methyl	1,6	*trans*	OH	$C(CH_3)CCCC$
cis-ortho-1,2-THC-C5	1,2	*cis*	C_5H_{11}	OH
cis-para-1,2-THC-C5	1,2	*cis*	OH	C_5H_{11}
trans-ortho-1,2-THC-C5	1,2	*trans*	C_5H_{11}	OH
trans-para-1,2-THC-C5	1,2	*trans*	OH	C_5H_{11}
cis-ortho-HHC	—	*cis*	C_5H_{11}	OH
cis-para-HHC	—	*cis*	OH	C_5H_{11}
trans-ortho-HHC	—	*trans*	C_5H_{11}	OH
trans-para-HHC	—	*trans*	OH	C_5H_{11}

TABLE III

STRUCTURES OF SYNTHETIC iso-THCs



Compound	*Double-bond*	*Position of H atoms at C3 and C4*	R_1	R_2
cis-ortho-8,9-iso-THC-C5	8,9	*cis*	OH	C_5H_{11}
cis-para-8,9-iso-THC-C5	8,9	*cis*	C_5H_{11}	OH
trans-ortho-8,9-iso-THC-C5	8,9	*trans*	OH	C_5H_{11}
trans-para-8,9-iso-THC-C5	8,9	*trans*	C_5H_{11}	OH

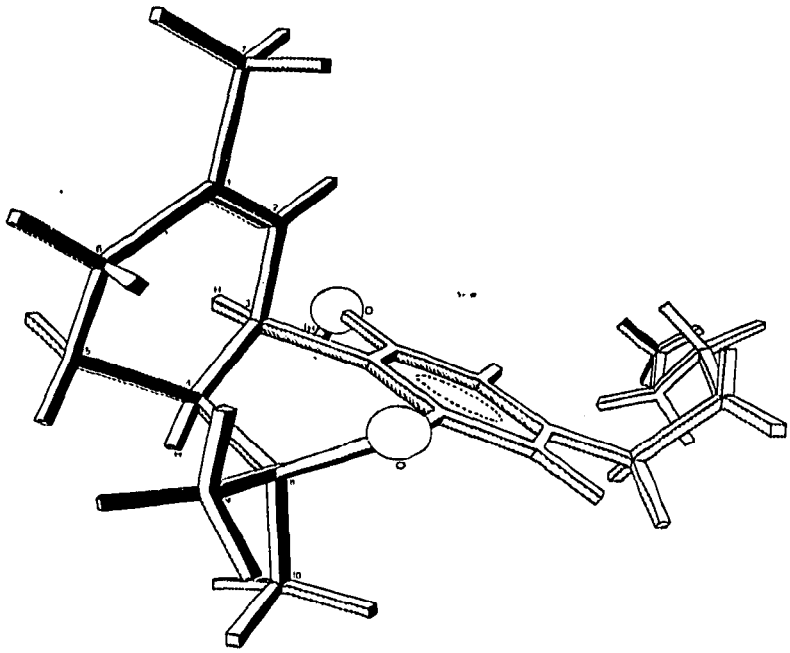


Fig. 2. *cis*-(3,4)-*para*-1,2-THC-C5.

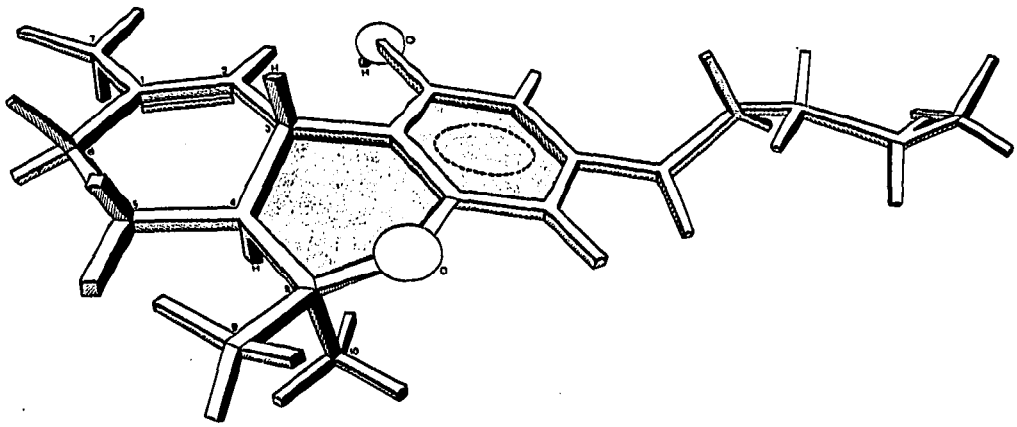


Fig. 3. *trans*-(3,4)-*para*-1,2-THC-C5.

Gas chromatography–mass spectrometry

An LKB 9000 instrument was used, with the following conditions:
Oven temperature 200°; separator 230°; ion source 290°.
Helium flow-rate 20 ml/min.
Ionisation potentials: for gas chromatography 20 eV; for mass spectrometry 20–10 eV (ref. 7).
Acceleration potential 3.5 kV; trap current 60 μA.
The spectra obtained are given in Tables IV–VII.

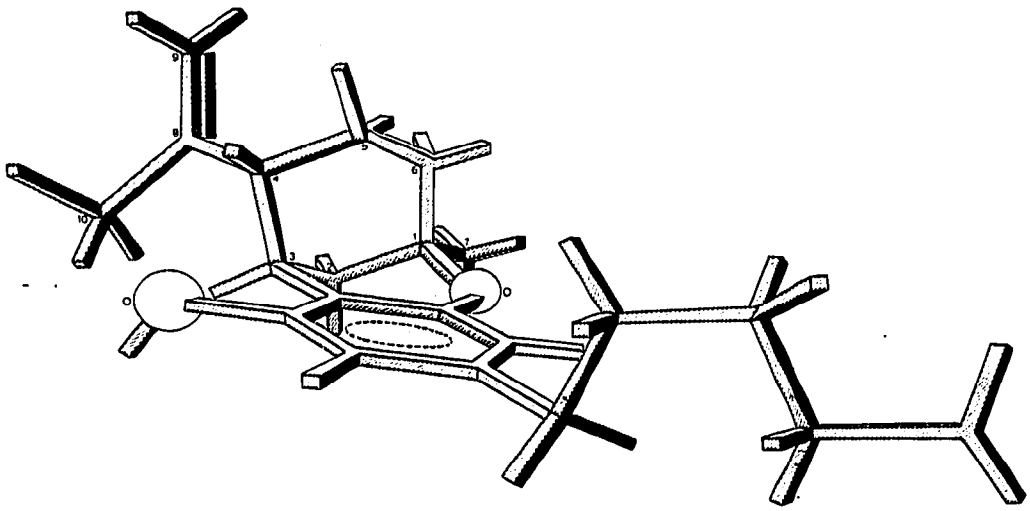


Fig. 4. *trans*-(3,4)-*para*-iso-8,9-THC-C5.

TABLE IV

PARTIAL MASS SPECTRA OF *cis–trans* ISOMERS OF *ortho*- AND *para*-1,6-THC-C5 AT 20 eV

Fragment	*m/e*	*cis-ortho*	*cis-para*	*trans-ortho*	*trans-para*
M	314	38	30	100	100
M-15	299	6	5	15	13
M-43	271	6	5	60	25
M-56	258	6	5	27	40
M-68	246	1	1	10	17
	243	0	0	2	5
	231	100	100	65	60

TABLE V

PARTIAL MASS SPECTRA OF *cis–trans* ISOMERS OF *ortho*- AND *para*-1,2-THC-C5 AT 20 eV

Fragment	*m/e*	*cis-ortho*	*cis-para*	*trans-ortho*	*trans-para*
M	314	85	65	100	100
M-15	299	10	10	55	63
M-43	271	100	100	32	30
M-56	258	7	10	25	27
M-68	246	12	11	10	5
	243	1	1	10	20
	231	11	12	72	40

TABLE VI

PARTIAL MASS SPECTRA OF ISO-8,9-THC AND ISO-8,9-THC-C5-α-METHYL (*trans-ortho–para*) AT 20 eV

Fragment	*m/e*	*ortho*	*para*	*ortho-α-Methyl*	*para-α-Methyl*
M	314 (328)	100	100	80	82
M-15	299 (313)	10	15	7	7
M-43	271 (285)	12	10	10	5
M-56	258 (272)	80	60	100	100
M-68	246 (260)	32	45	18	25
	243 (257)	5	5	5	10
	231 (245)	22	18	15	25

TABLE VII

PARTIAL MASS SPECTRA OF *cis–trans* ISOMERS OF *ortho-* AND *para*-1,6-THC-C5-α-METHYL AT 20 eV

Fragment	*m/e*	*cis-ortho*	*cis-para*	*trans-ortho*	*trans-para*
M	328	75	100	15	100
M-15	313	20	10	2	7
M-43	285	20	90	5	20
M-56	272	25	45	25	90
M-68	260	75	10	1	1
	257	5	5	1	1
	245	100	10	10	70
	231	70	35	100	10

RESULTS AND DISCUSSION

From the known chemical pathways in the synthesis of cannabinoids (Fig. 1), it can be predicted which side-products can possibly be formed. Furthermore, the well known behaviour of cannabinoids in gas chromatography[7] and mass spectrometry[2,3,8–11] enables the retention times and fragmentation patterns of the synthetic products to be rationalized in advance.

In a very specific synthesis of *trans*-1,2-THC, it is always probable that the *cis*-1,2-THC is also formed, although in small amounts[2,3]. In general, *cis–trans* isomerisation influences the shape of the molecule and it is often observed that the *cis*-isomer is more compact than the corresponding *trans*-isomer.

With the *cis–trans* isomers of THC, it has been observed that the *cis*-isomer has a more compact (spherical) configuration than the *trans*-isomer, as the cyclohexenyl ring is in a plane perpendicular to the plane of the pyran ring (see Figs. 2 and 3). This might imply that the *cis*-isomer has a shorter retention time than the *trans*-THC, owing to a decreased interaction with the stationary phase. Similar behaviour was found for the cannabinoids with an *ortho*-substituted side-chain and for those with different lengths of side-chain[7]. An increase in the length of the side-

chain resulted in an increased retention time, which might be due to an increase in the surface area of the molecule that is available for interaction.

The same effect was observed for the *ortho*-substituted side-chain. In this instance, the length of the side-chain, shielded by the cyclohexenyl ring, is less available for interaction with the stationary phase. When a reaction mixture for 1,2-THC synthesis, for example, was submitted to GC–MS analysis, the side-products appeared to have shorter retention times than 1,6- and 1,2-THC and CBD (see Fig. 1 and Table I). The side-products were identified by means of mass spectrometry (Tables IV–VII), the retention times were measured and the fictive retentions of the compounds were calculated as described earlier[7] (relative to *trans-para*-1,2-THC-C5 with a fictive retention of 100). The results are given in Table I. The fictive retentions of the cannabinoids that occur in nature are also given in Table I and it can be concluded that the probability of identification of *cis-para*-1,2-THC-C5 in natural hashish strongly depends upon the efficiency of the separation technique.

When the mass spectrometric behaviour of two cannabinoids is very similar (Tables IV–VII), then gas chromatographic data can be used for further identification. From Table VIII it can be concluded that the ratio of the retention times of *ortho–para* isomers of the cannabinoids also applies to iso-THC and the *cis*-isomers of 1,2- and 1,6-THC.

TABLE VIII

RATIO OF THE FICTIVE RETENTIONS OF iso-, *cis*- AND *trans*-THC AND HHC WITH AN *ortho*- AND *para*-SUBSTITUTED PENTYL SIDE-CHAIN

Compound	*Ratio ortho/para*
trans-iso-8,9-THC-C5	1.20
trans-iso-8,9-THC-C5-α-methyl	1.21
cis-1,6-THC-C5	1.15
trans-1,6-THC-C5	1.28
cis-1,2-THC-C5	1.15
trans-1,2-THC-C5	1.39
cis-HHC	1.40
trans-HHC	1.37

As mentioned above, *cis*-THC has a folded structure (Fig. 2) and the cyclohexenyl ring is in a plane perpendicular to that of the pyran ring. It has been shown that this difference in structure between *cis*- and *trans*-THC isomers is reflected in the retention behaviour (Table IX). It can be shown that the retention time of the *cis*-THCs is shortened by a factor of 1.85 in comparison with those of *trans*-THCs.

In the synthesis of 1,2-THC, *para*-menthadienol is coupled with olivetol. In Fig. 1, it is shown that four isomers of CBD intermediates (*cis–trans-para*-CBD and *cis–trans-ortho*-CBD) can be obtained. Thereafter, the phenolic OH group attacks the double-bond either in the 8,9-position, which results in 1,2-THC, or in the 1,2-position, which results in iso-8,9-THC.

TABLE IX

RATIO OF THE FICTIVE RETENTIONS OF *cis–trans* ISOMERS OF 1,6- AND 1,2-THCs AND HHC

Compound	*Ratio cis/trans*
ortho-1,6-THC-C5	1.94
para-1,6-THC-C5	1.83
ortho-HHC	1.93
para-HHC	1.88
ortho-1,2-THC-C5	1.70
para-1,2-THC-C5	1.89
para-1,6-THC-C5-α-methyl	1.79

TABLE X

RATIO OF FICTIVE RETENTIONS OF THREE ISOMERIC THC MOLECULES WITH DIFFERENT STERIC CONFIGURATIONS

Compound	*Ratio of fictive retentions*		*Configuration*
	iso-THC–cis-1,2-THC	*iso-THC–trans-1,2-THC*	
para-1,2-THC	2.62	4.95	sphere I: sphere II sphere I: plane
ortho-1,2-THC	2.72	4.65	sphere I: sphere II sphere I: plane

From Figs. 2 and 4, it can be concluded that both *cis*-1,2-THC and iso-8,9-THC are somewhat spherical molecules, and from Fig. 3 that *trans*-1,2-THC is a "stretched" molecule.

These steric differences are also reflected in the gas chromatographic behaviour. From Table X, it can be shown that the ratio of the retention times of iso-THC to those of *cis*-1,2-THC is constant and independent of the *ortho–para* substitution of the pentyl side-chain (sphere I: sphere II = iso-:*cis*-THC). Also, the ratio of the retention times of iso-THC to those of *trans*-1,2-THC is constant and independent of the *ortho–para* substitution of the side-chain (sphere I: plane = iso-:*trans*-THC). From these isomeric THCs with different steric configurations, the conclusion can be drawn that these configurations play an essential role in the interaction of cannabinoids with the stationary phase in the column.

CONCLUSION

Information about the identity of the side-products in a particular synthesis can be obtained by means of gas chromatography. Full identification of the structures of the compounds is obtained from the combined data of gas chromatography and mass spectrometry. The possibility of isolating cannabinoids with the hydrogen atoms in the 3- and 4-positions in the *cis*-configuration is strongly limited by the separation efficiency, but cannot be neglected.

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