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# MINOR COMPONENTS OF CANNABIS RESIN

## V. MASS SPECTROMETRIC DATA AND GAS CHROMATOGRAPHIC RETENTION TIMES OF CANNABINOID COMPONENTS WITH RETENTION TIMES SHORTER THAN THAT OF CANNABIDIOL

LARS STRÖMBERG

*The National Laboratory of Forensic Science, (Statens kriminaltekniska laboratorium), Fack, S-171 20 Solna 1 (Sweden)*

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SUMMARY

Minor cannabinoid components of cannabis resin were analyzed by gas chromatography and mass spectrometry. Complete mass spectra were recorded for four such components. Two of these components were found to be cannabidivarol and cannabicyclol, while the other two were isomers of cannabidiol but were eluted before cannabicyclol, in contrast to hitherto described natural isomers of cannabidiol. The relative retention times of eight cannabinoids, including the major ones, were determined on three different stationary phases.

## INTRODUCTION

In Parts I and II[1,2] of this series, the gas chromatographic separation of minor components of a hashish extract was described. Mass spectrometric data indicated that some of these components were terpenic compounds[2,3], in agreement with earlier investigations. Among the minor components of cannabis resin there are also isomers and homologues of the major cannabinoids cannabidiol (CBD), $\Delta^{1,2}$-tetrahydrocannabinol ($\Delta^{1,2}$-THC) and cannabinol (CBN), as demonstrated by Vree *et al.*[4] and others. In this paper, the mass spectrometric data and gas chromatographic retention times of some additional components of this type are reported and discussed.

## EXPERIMENTAL

The instrument used for combined gas chromatography–mass spectrometry (GC–MS) was an LKB 9000. An IBM 1800 computer was used for the calculations. The inlet system (separator) and ion source temperatures were about 280 and 300°, respectively. The electron energy was 70 eV.

The column used for GC–MS was a 4.5-m glass tube of O.D. 6 mm (0.25 in.) and I.D. 2 mm with a coil diameter of 100 mm, packed with Gas-Chrom Q (60–80

mesh), coated with 3% OV-101 methyl silicone. The column temperature was programmed from 130 to 200° at 4°/min. The flow-rate of carrier gas (helium) was about 30 ml/min.

For the study of retention times, the gas chromatograph used was a Perkin-Elmer F11 with a No. 4 analyzer unit (all-glass system and flame ionization detector). The columns were 1.9-m glass tubes of O.D. 6 mm (0.25 in.) and I.D. 2 mm with a coil diameter of 130 mm. The injection temperature was about 220° and the flow-rate of carrier gas (nitrogen) was about 30 ml/min. The retention times were determined under isothermal conditions at the temperatures and on the liquid phases listed in Table III.

The hashish (the same material as previously investigated[1–3]) was extracted according to the procedure previously described[2]. The extract was analyzed by GC–MS.

## RESULTS AND DISCUSSION

### *Complete mass spectra*

The MS data were obtained by analysis of the six fractions corresponding to the numbered GC peaks in Fig. 1, which corresponds to part of a previously published chromatogram (ref. 3, Fig. 1). Complete spectra were recorded for fractions 51, 52, 53 and 54 and these spectra are shown in Figs. 2–5. The ten peaks of highest intensity observed in the spectra of fractions 51, 52 and 53 are listed in Table I. The following comments can be made.

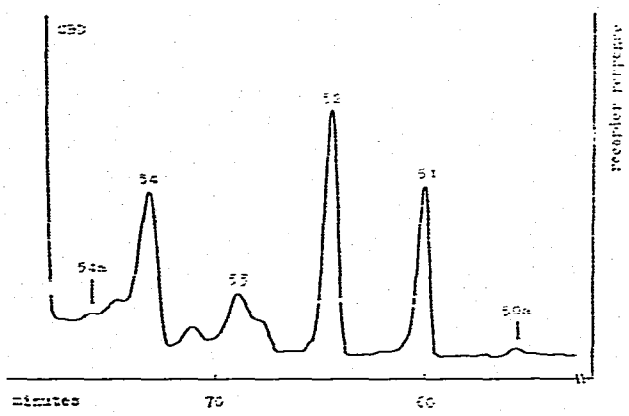


Fig. 1. Total ionization current chromatogram of minor cannabinoid components with retention times shorter than that of CBD on the OV-101 column. Mass spectrometric data and relative retention times are reported for the fractions numbered in the chromatogram. The peaks of the terpenic components eluting before 40 min are not shown.

*Fraction 51.* The mass spectrum of this fraction (Fig. 2) shows a parent peak at *m/e* 286. The fragmentation pattern indicates that the fraction consists essentially of the CBD-C3 homologue cannabidivarol, as five of the major peaks of this spectrum and the CBD-C5 spectrum[5] differ from each other by 28 mass units (*m/e* 286, 218,

TABLE I

MASS SPECTROMETRIC DATA OBTAINED FOR FRACTIONS 51–54 AND FOR THE CANNABICYCLOL STANDARD

For fractions 51–53 and cannabicyclol the ten highest intensities are listed, and for fraction 54 the ten intensities corresponding to those listed for cannabicyclol. The *m/e* values of the parent peaks are printed in bold.

| *Fraction No. (Fig. 1)* | *m/e values* / *Relative intensities* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 51 | 203 | 218 | 204 | 41 | 174 | 165 | 43 | 121 | 91 | **286** |
| | 100 | 20 | 15 | 12 | 11 | 11 | 9 | 8 | 7 | 6 |
| 52 | 231 | **314** | 232 | 233 | 41 | 299 | 271 | 315 | 258 | 174 |
| | 100 | 22 | 19 | 11 | 10 | 8 | 8 | 6 | 6 | 6 |
| 53 | 231 | 232 | 43 | 41 | 217 | 174 | **314** | 245 | 243 | 230 |
| | 100 | 21 | 21 | 18 | 12 | 10 | 8 | 5 | 5 | 5 |
| 54 | 231 | 41 | 232 | 174 | 43 | 69 | 55 | 81 | **314** | 299 |
| | 100 | 31 | 16 | 15 | 9 | 8 | 7 | 6 | 3 | 2 |
| Cannabicyclol | 100 | 9 | 18 | 11 | 4 | 4 | 3 | 3 | 5 | 3 |

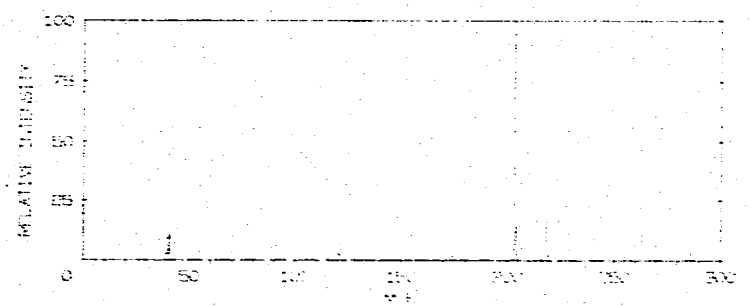

Fig. 2. Mass spectrum of fraction 51.

204, 203 and 165 for fraction 51 and *m/e* 314, 246, 232, 231 and 193 for CBD-C5). The retention time study also supports this assumption (see below). Cannabidivarol (cannabidivarin) was found in hashish by Vollner *et al.*[6], who reported major peaks at *m/e* 286, 271, 218, 203, 174 and 165.

*Fractions 52 and 53.* In the mass spectra of these fractions (Figs. 3 and 4), the parent peaks lie at *m/e* 314. The base peaks at *m/e* 231 presumably represent the chromenyl ion[7], a typical fragment of cannabinoid molecules having a mol. wt. of 314 and carrying a pentyl side-chain (refs. 5, 7, 8 and others).

Other peaks, typical of this type of cannabinoid[5-7], are also present in both spectra, *e.g.*, at *m/e* 299, 271, 258, 246, 243, 193 and 174, indicating that fractions 52 and 53 consist essentially of compounds structurally related to CBD and THC.

It is known that the structure of the alicyclic ring system strongly influences the mass spectral fragmentation of the different types of cannabinoids[4-7]. In the mass spectra of fractions 52 and 53, the intensities at *m/e* 246 are low, which indicates that

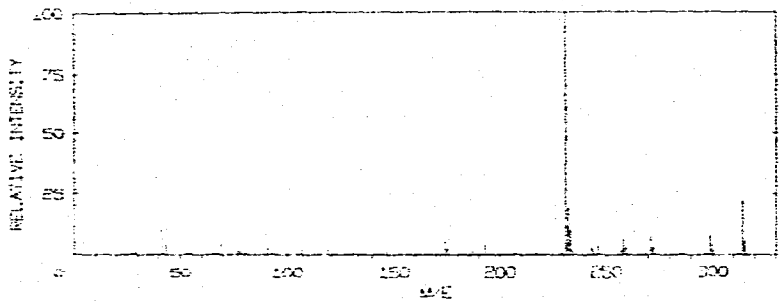


Fig. 3. Mass spectrum of fraction 52.

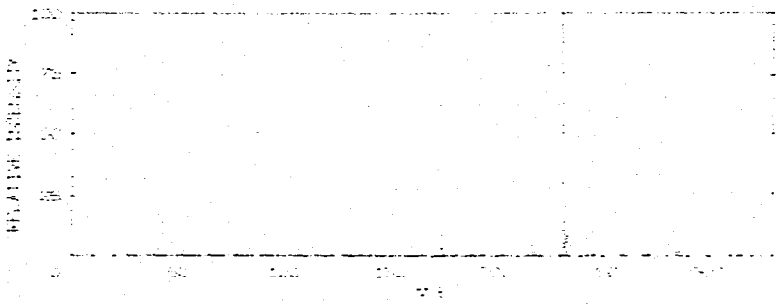

Fig. 4. Mass spectrum of fraction 53.

the constituents of these fractions are not subject to *retro*-Diels-Alder degradation to such an extent as CBD and $\Delta^{1,6}$-THC (refs. 5 and 7). Further, the mass spectra of fractions 52 and 53 both show small peaks at *m/e* 299. According to Claussen *et al.*[7], this may exclude some alternatives concerning the position of the double bond in the alicyclic ring.

*Fraction 54.* The mass spectrum of this fraction is shown in Fig. 5. The parent peak lies at *m/e* 314 and the base peak at *m/e* 231. Fraction 54 has the same retention time as cannabicyclol on the OV-101 column (see below). The ten strongest peaks of the cannabicyclol standard mass spectrum and the corresponding peaks of the spectrum of fraction 54 are compared in Table I. Fig. 5 shows an additional peak at

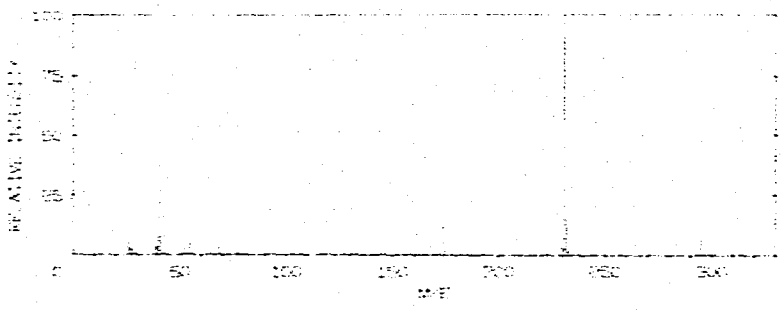

Fig. 5. Mass spectrum of fraction 54.

*m/e* 295, which does not originate from cannabicyclol and is presumably due to another compound eluted in the same fraction. This compound can be neither CBD nor "fraction 53", as the mass spectra of these show no appreciable peaks at *m/e* 295.

*Mass fragmentography*

In order to detect other minor cannabinoid components with a mol. wt. of 314, mass fragmentographic (MF) analyses were carried out at *m/e* 314 and 231 under the conditions mentioned under Experimental. In the two mass fragmentograms obtained, peaks were observed at retention times corresponding to those of fractions 50a, 52, 53, 54 and 54a shown in Fig. 1. This result demonstrates the presence of peaks at these *m/e* values in the mass spectra of the fractions mentioned. The signal-to-noise ratio was better than 5:1 for the lowest MF peak.

In the sample used, the amounts of fractions 50a and 54a were too small for their complete mass spectra to be recorded. However, conclusions concerning the peak heights at *m/e* 231 and 314 in these spectra can be drawn from the MF analyses. The occurrence of major MF peaks at these *m/e* values is typical of cannabinoids having a mol. wt. of 314 and carrying a pentyl side-chain[5,7,8].

In the MF analyses, the corresponding total ionization current (TIC) peak heights obtained varied owing to different sample sizes and imperfect reproduction of the column temperature programme. Therefore, the MF peak heights obtained were normalized by dividing them by the heights of the corresponding TIC peaks. The set of normalized MF peak heights corresponding to *m/e* 314 was then multiplied by an attenuation factor, as the MF analyses were carried out at different instrument sensitivities. The two MF peak heights of a particular fraction thus calculated now correspond to the same TIC signal and the same sensitivity as in a conventionally recorded mass spectrum. The normalized MF peak heights are listed in Table II and are denoted by $h_{314}$ and $h_{231}$. In the last column of Table II the ratios $h_{314}/h_{231}$ are expressed as percentages. The ratios for fractions 52, 53 and 54 then correspond directly to the intensities at *m/e* 314 in the complete spectra of these fractions, as these spectra are all normalized by setting the intensities at *m/e* 231 equal to 100%.

TABLE II

MASS FRAGMENTOGRAPHIC DATA OBTAINED FOR FRACTIONS 50a, 52, 53, 54 AND 54a
The *h* values were obtained by dividing the mass fragmentographic peak heights by the heights of the corresponding total ionization current peaks.

| *Fraction No. (Fig. 1)* | $h_{231}$ | $h_{314}$ | $h_{314}/h_{231} \cdot 100$ |
|---|---|---|---|
| 50a | 1 | 0.5 | 50 |
| 52 | 8 | 1.6 | 20 |
| 53 | 10 | 0.8 | 8 |
| 54 | 13 | 0.44 | 3.4 |
| 54a | 6 | 4 | 66.7 |

As already mentioned, the *h* values in Table II were obtained by dividing MF peak heights by the corresponding TIC peak heights. The TIC peak height may at least be regarded as an approximate measure of the maximum total ion beam intensity and consequently of the sum of all the peaks of the corresponding mass spectrum.

Therefore, the *h* value is a measure of the height of one peak relative to the sum of all the peaks of the actual spectrum. Thus, the high intensities of the *m/e* 231 peaks in the spectra of fractions 52, 53 and 54 correspond to $h_{231}$ values of about 10, as seen in Table II.

These results indicate that the mass spectrum of fraction 54a has its base peak at *m/e* 231 and a major peak at *m/e* 314, and this fraction may therefore consist essentially of a cannabinoid of the actual type. The *h* values of fraction 50a, on the other hand, indicate that its mass spectrum has only minor peaks at *m/e* 231 and 314. Cannabinoids of the actual type should therefore not be present in appreciable amounts in this fraction. This assumption is supported by the low retention time, which is lower than that of the C3 homologue in fraction 51.

Further information concerning fractions 50a and 54a may be obtained by analysis of cannabis materials that contain larger amounts of these fractions. The existence of such materials does not seem unlikely; it is known that the chemical composition of different cannabis materials varies considerably.

*Gas chromatographic retention times*

By addition of cannabicyclol to the hashish extract, it was found that it had the same retention time as fraction 54 on the OV-101 column. This result supports the MS identification of this fraction with cannabicyclol.

In order to study the retention times of fractions 51–54 on different liquid phases, these fractions were isolated from the hashish extract using a semi-preparative OV-101 column and a temperature programme not very different from that employed in the GC–MS analysis (*cf.* Fig. 1). The fractions were easily identified by virtue of their elution order and relative peak heights.

The fractions thus collected were then added to samples of the original hashish extract in such amounts that their concentrations were increased three to five times. When gas chromatograms of these mixtures were compared with that of the original extract alone, the enhanced peaks could easily be recognized, permitting the determination of relative retention times. Such experiments were carried out using columns with three different liquid phases (OV-101, OV-17 and Dexsil 300) under the conditions mentioned under Experimental. The results are given in Table III. The following comments can be made.

*Retention times on OV-101 (methyl silicone).* The experiments with the OV-101 column showed that the retention times of the isolated fractions were the same as in the original extract. This result demonstrates the stability of the fractions under the conditions used in the isolation process. As seen from Table III, the OV-101 column is superior to the other columns for separating fractions 52, 53 and 54 from each other.

*Retention times on OV-17 (a 50% phenyl-substituted methyl silicone).* As seen from Table III, a retention time ratio of 69/36 = 1.92 for CBD and fraction 51 was obtained on the OV-17 column at 215°. Correspondingly, Vree *et al.*[9] obtained a ratio of 2.04 for CBD and cannabidivarol (CBD-C3) on OV-17 at 200°. This result supports the assumption from the mass spectrometric analysis that fraction 51 consists essentially of cannabidivarol.

Table III shows that, on the OV-17 column, fractions 52 and 53 are both eluted before cannabicyclol, in contrast to the known natural cannabinoids with a mol. wt.

TABLE III

RELATIVE GAS CHROMATOGRAPHIC RETENTION TIMES OBTAINED FOR FRACTIONS 51–54, CANNABICYCLOL, CANNABICHROMENE AND THE MAJOR CANNABINOIDS

The values for cannabichromene were estimated by extrapolation of those obtained at higher column temperatures. $\Delta^{1,6}$-THC was eluted after CBD on all three columns.

| *Liquid phase* | *Relative retention times ($\Delta^{1,2}$-THC = 100)* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *Fraction 51* | *Fraction 52* | *Fraction 53* | *Fraction 54 and cannabicyclol* | *CBD* | *Cannabichromene* | *$\Delta^{1,2}$-THC* | *CBN* |
| 3% OV-101 at 183 | 34 | 42 | 51 | 60 | 72 | 75 | 100 | 126 |
| 3% OV-17 at 215 | 36 | 39 | 52 | 54 | 69 | 66 | 100 | 129 |
| 3% Dexsil 300 at 183 | 28 | 41 | 46 | 52 | 62 | 65 | 100 | 128 |

of 314, *i.e.*, cannabichromene, CBD, $\Delta^{1,6}$-THC and $\Delta^{1,2}$-THC. The short retention times of fractions 52 and 53 may give information about their structure. Vree *et al.*[4] found that the structure of the aromatic ring system of cannabinoids strongly influences their retention times. They showed, for example, that the retention times on OV-17 of $\Delta^{1,2}$-THC-C5 and $\Delta^{1,6}$-THC-C5 are about 1.3 times longer than those of their respective *ortho*-isomers (in which the pentyl and hydroxyl groups on the aromatic ring are interchanged). Table III shows that this relationship was also obtained in this study for CBD and fraction 53. Provided that the structure of the aromatic ring system influences the retention time of CBD in a similar manner to that of the THCs, the relationship indicates that fraction 53 may consist essentially of *ortho*-CBD. However, this compound, which has the same alicyclic ring system as CBD itself, should undergo *retro*-diene degradation, leading to a peak at *m/e* 246 of considerable intensity in its mass spectrum. As pointed out earlier, such a peak is not observed in the mass spectrum of fraction 53 (Fig. 4).

If the cannabinoids in fractions 52 and 53 are structurally related to the known cannabinoids, the question of whether their heterocyclic rings are closed or open arises. Unfortunately, the effect of this ring closure on the retention time of an unknown cannabinoid does not seem to be predictable. For example, consider the conversion of cannabigerol into cannabichromene. This ring closure causes a considerable decrease in the retention time[1]. This process may be compared with the conversion of dihydro-(8,9)-CBD into $\Delta^{1,2}$-THC via CBD. The first step of this process causes a decrease of about 7% in the retention time[1]. The second step, conversion of CBD into $\Delta^{1,2}$-THC, increases the retention time, and this increase is much larger than 7% (*cf.*, Table III) so that the net result from the ring closure of dihydro-(8,9)-CBD is a considerable increase in the retention time. Both of the above processes are ring closures including alkylation of hydroxyl groups, leaving the number of double bonds unchanged. Nevertheless, they strongly influence the retention times in opposite directions. It is true that the first process also involves a change in the position of a double

bond, but it seems unlikely that this is the complete explanation of the reversal of the effect on the retention times.

Vree *et al.* analyzed Lebanese hashish by gas chromatography on OV-17 at 200° (ref. 9, Fig. 15). When examining their gas chromatogram, it seems that peak 4 has a retention time of about 1.1 relative to cannabidivarol. It is stated that this peak represents a mixture of a compound of mol. wt. 314 and CBN-C1. In the present study, the ratio of the retention times of fraction 52 and cannabidivarol (fraction 51) was 39/36 = 1.1 on the OV-17 column at 215°, as seen from Table III. The compound found by Vree *et al.* may thus be identical with the major constituent of fraction 52. As far as CBN-C1 is concerned, it does not seem to interfere in the MS analysis of fraction 52 (Fig. 3), as no appreciable intensity is observed at *m/e* 239, the base peak in the mass spectrum of CBN-C1 (ref. 9).

*Retention times on Dexsil 300 (polycarborane siloxane).* Dexsil 300 was used in an earlier study[2] in the gas chromatography of the heavy minor components of cannabis resin, *i.e.*, those eluted after CBN. As seen in Table III, Dexsil 300 gives the best separation of fractions 51 and 52 while OV-101 is superior to Dexsil 300 in separating fractions 52, 53 and 54 from each other. Dexsil 300 also gives an excellent separation of CBD, $\Delta^1$-THC and CBN and can be recommended for the routine determination of these cannabinoids.

To summarise, the mass spectra and the retention times of fractions 51 and 54 strongly indicate that these fractions consist essentially of cannabidivarol and cannabicyclol, respectively. The MF data indicate that fraction 54a may consist essentially of a cannabinoid with a mol. wt. of 314 and a pentyl side-chain, whereas this is presumably not the case for fraction 50a. The mass spectra of fractions 52 and 53 indicate that these fractions consist essentially of cannabinoids, structurally related to CBN and THC, having a mol. wt. of 314 and carrying a pentyl side-chain. Some conclusions concerning the structure of their alicyclic and aromatic ring systems can be drawn from the mass spectra and the retention times but the structures cannot be fully elucidated at present.

In the present series of papers, MS data have been reported only for minor components eluted before CBD on methyl silicone columns. Little is known about the minor components of cannabis that are eluted after CBN. The presence of such components in marihuana cigarette smoke was demonstrated in an earlier paper[1]. Conventional GC–MS analysis of these components is a difficult task because liquid phases stable at 350–400° would be needed. Although good gas chromatograms can be obtained with Dexsil 300 columns, bleeding interferes severely in the GC–MS analysis. However, preliminary experiments have indicated that MS data may be obtained by MF or by the method of continuous scanning of the GC column eluates of these components.

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