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MINOR COMPONENTS OF CANNABIS RESIN

VI. MASS SPECTROMETRIC DATA AND GAS CHROMATOGRAPHIC RETENTION TIMES OF COMPONENTS ELUTED AFTER CANNABINOL

LARS STRÖMBERG

Statens kriminaltekniska laboratorium (The National Laboratory of Forensic Science), Fack, S-581 01 Linköping (Sweden)

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SUMMARY

Minor components of cannabis resin with longer retention times than that of cannabinol have been analyzed by use of a combined gas chromatograph–mass spectrometer–computer system. The presence of 30 such components has been detected. The data obtained indicate that some of the components are hydroxy derivatives of cannabinoids related to olivetol.

INTRODUCTION

In Part I of this series[1] the gas chromatographic (GC) analysis of minor components of a hashish extract was described. The light minor components, *i.e.*, those eluted before cannabidiol (CBD) on methyl silicone columns, were analyzed by combined GC–mass spectrometry (MS). The results of this study were reported in Parts II, IV and V of this series[2–4]. This paper concerns the GC–MS analysis of the heavy minor components, *i.e.*, those eluted after cannabinol (CBN). GC analyses suggest that such components are generally present in cannabis resins and also in cannabis smoke condensates[1].

EXPERIMENTAL

The instrument used in this study was a LKB 2091 gas chromatograph–mass spectrometer connected to a LKB 2130 GC–MS data analysis system. The analyses were carried out by the method of continuous scanning with a scan cycle time of *ca.* 5 sec and a mass range of 10–500 a.m.u. The temperatures of the separator and ion source were *ca.* 260 and 270° respectively. The electron energy was 70 eV. The GC column used was a glass tube (2.5 m × 6 mm O.D. × 2 mm I.D.; coil diameter, 175 mm) packed with Gas-Chrom Q (80–100 mesh) which was coated with 1% OV-17 (a 50% phenyl-substituted methyl silicone). The carrier gas (helium) flow-rate was 30 ml/min, and the injector temperature was 245°. The column temperature was

initially 195°, and after 8 min it was programmed at 2°/min to 250°. The GC–MS experiments were carried out at the LKB application laboratory in Stockholm.

For the determination of retention times, a Perkin-Elmer F11 gas chromatograph was used with a No. 4 analyzer unit (all-glass system and flame ionization detector). The column was a glass tube (1.9 m × 6 mm O.D. × 2 mm I.D.; coil diameter, 130 mm) packed with Gas–Chrom Q (80–100 mesh) which was coated with 1% OV-17. The carrier gas (nitrogen) flow-rate was 30 ml/min, the column temperature was 190° and the injector temperature was *ca.* 250°.

The hashish (the same material as previously investigated[1–4]) was extracted according to the procedure described earlier[2]. The extract was analyzed by GC–MS.

RESULTS AND DISCUSSION

Mass spectrometry

As pointed out in Part V[4], good gas chromatograms of the heavy cannabis resin components are obtained by using columns of Dexsil 300 polycarborane siloxane. However, this liquid phase caused severe interference in the GC–MS analysis by contaminating the ion source. Therefore, OV-17 columns were used in this study. The column bleeding could be limited by the use of a rather short column. In combination with a low liquid-phase load, this permitted analysis below 250°. With this chromatographic system, some components were only partially resolved, giving mixed spectra in some cases. This will be further discussed below.

The MS data were obtained by continuous scanning of the fractions corresponding to the GC peaks shown in Fig. 1, which is a computer reconstruction of the total ion current (TIC) chromatogram. A preliminary examination of the collected

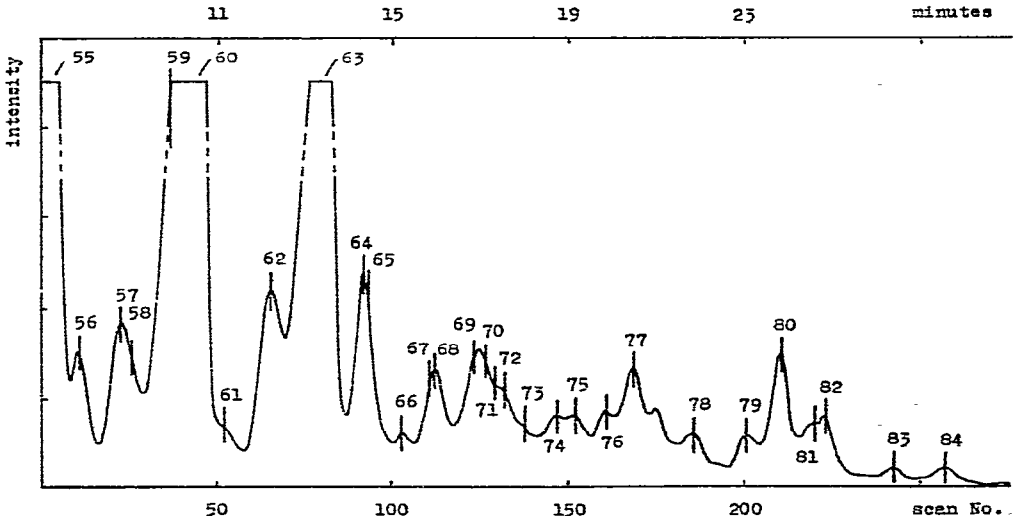

Fig. 1. Computer reconstruction of a TIC chromatogram of the heavy minor components of the hashish extract. The vertical bars indicate the positions of the mass chromatographic peaks observed in the study. The peak numbering is a continuation of the one introduced in Parts II, IV and V of this series[2–4]. Fractions 55, 60, 62 and 63 contain CBD, $\Delta^{1,2}$-THC, CBG and CBN, respectively, as the main constituents. The GC peaks corresponding to the light minor components are not shown.

information showed the presence of peaks at *m/e* 193 and 231 in many of the mass spectra. These peaks occur in the mass spectra of cannabinoids such as CBD and $\Delta^{1,2}$-tetrahydrocannabinol ($\Delta^{1,2}$-THC) which are related to olivetol[5]. Peaks at *m/e* 247 were also present in some spectra. This peak is present in the mass spectrum of cannabigerol (CBG) which also belongs to the "olivetol cannabinoids" (see ref. 5 for the structure of the corresponding fragment ions). The occurrence of these peaks in the mass spectra of some components is shown by the mass chromatograms in Fig. 2. These observations indicate that some of the components are structurally related to CBD and CBG.

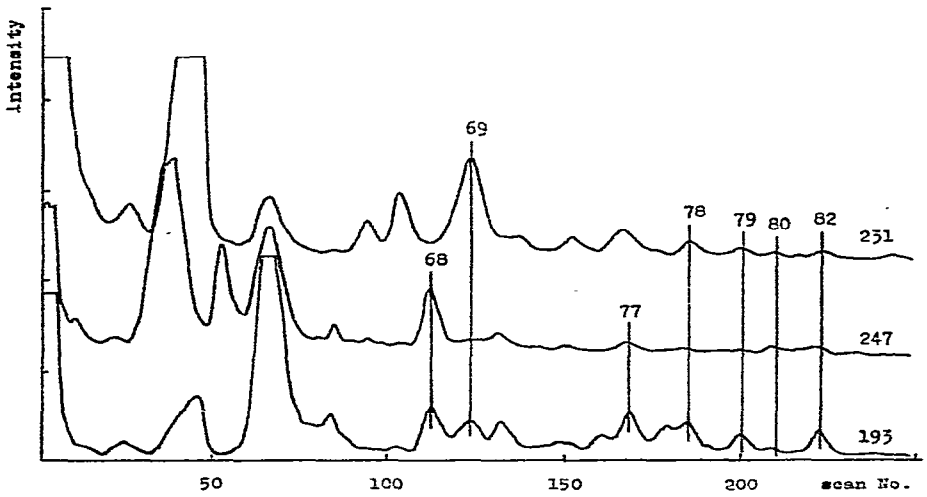

Fig. 2. Mass chromatograms at 193, 231 and 247 a.m.u.

Compounds related to the main cannabinoids, CBD, $\Delta^{1,2}$-THC and CBN, have been found in cannabis by many workers, and this field was recently reviewed by Hanůs and Krejčí[6]. Examples of such compounds are side-chain homologues, hydroxy and carboxy derivatives and methyl ethers of CBD, THC, CBN and CBG. In general, carboxy derivatives must be silylated prior to GC analysis in order to prevent decarboxylation, and they were not studied in this investigation.

In order to investigate whether some of the components studied were hydroxy derivatives or higher homologues of CBD and CBG or their isomers as indicated by the relatively long retention times, mass chromatograms were constructed at 314 and 316 a.m.u. (*i.e.* the molecular weights of CBD and CBG) to which masses of 16 and 14 a.m.u. (corresponding to introduction of hydroxyl groups and to extension of the carbon skeleton by methylene groups, respectively) were added. The mass chromatograms obtained indicated that some components could indeed be hydroxy derivatives of the type concerned, whereas higher homologues were not detected. As an example, the mass chromatograms at 332 and 348 a.m.u. are shown in Fig. 3.

As pointed out above, the GC resolution was partially incomplete. Therefore, mixed mass spectra were obtained in some cases. However, this effect could be compensated for to some extent by constructing mass chromatograms, *i.e.* graphs of in-

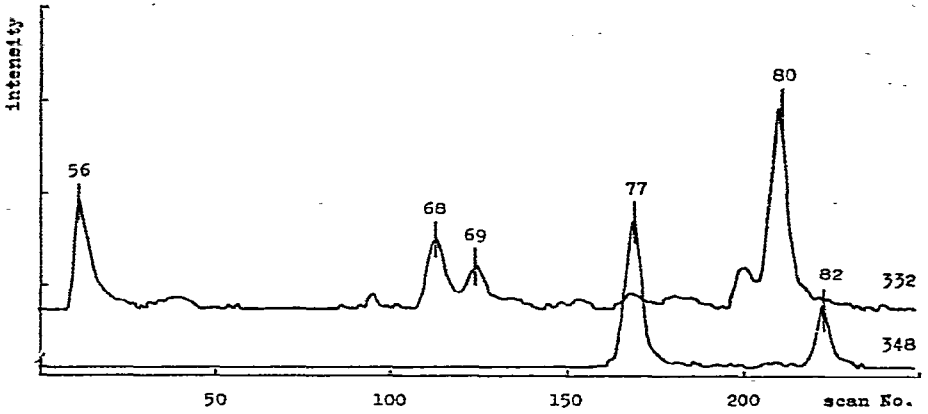

Fig. 3. Mass chromatograms at 332 and 348 a.m.u.

tensity *versus* retention time. This method permits the MS peaks to be associated with precise retention times. Thus, Fig. 4 shows the mass chromatograms at 231, 313, 328 and 330 a.m.u. together with a part of the TIC chromatogram surrounding component 69. Although the exact mass spectra of all of the individual components could not be determined in this way, conclusions concerning their nature could be drawn from the information obtained as discussed below.

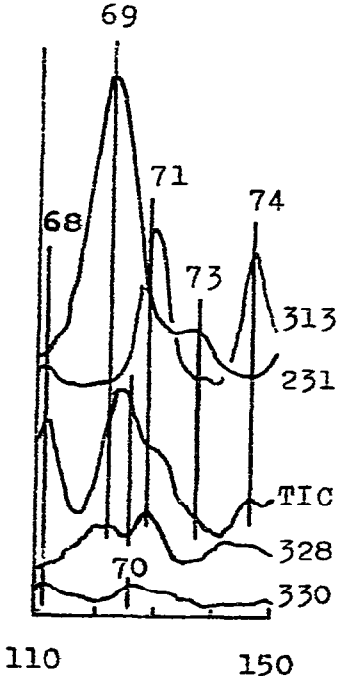

Fig. 4. Mass chromatograms at 231, 313, 328 and 330 a.m.u. together with the TIC chromatogram between scans 110 and 150.

A mass spectrum of fractions 64 and 65 (*cf.* Fig. 1) showed the typical fragmentation pattern of a long-chain alkane. Such alkanes were found in cannabis by De Zeeuw *et al.*[7] who also determined their relative retention times. The retention time observed in this study (2.0 relative to CBD) suggests that the corresponding constituent may be *n*-triacontane. A mass spectrum of fraction 67 was very similar to that of CBN. A CBN isomer having this property, cannabinodiol (CBND), was found in cannabis by Van Ginneken *et al.*[8]. CBND should therefore be a main constituent of the fraction concerned. This is further supported by retention data (see below).

The main constituents of fractions 68, 69, 77, 78, 79, 80 and 82 constitute a group having molecular weights which differ from those of CBD or CBG by 16 or 32 a.m.u., corresponding to one or two additional oxygen atoms. Some typical properties of the mass spectra of these components are shown in Table I. The presence of peaks at M−2, M−15, M−18 and M−33 indicates a structural relation with terpenic alcohols (see ref. 3 and literature cited there). On the other hand, the occurrence of peaks at 193, 231 and 247 a.m.u. indicates the presence of an olivetolic moiety. Therefore, it seems likely that the components discussed are hydroxyl derivatives of CBD and CBG or of their isomers. Since fragmentation of cannabinoids takes place predominantly in the non-aromatic terpenic moiety[9], the additional hydroxyl groups should be located in that part of the molecules.

TABLE I

MASS SPECTRAL FRAGMENTATION OF COMPONENTS 68, 69, 77, 78, 79, 80 AND 82

Observed peaks are indicated by × (peak heights below 1% are indicated by (×)). The peaks at M−15, M−18, M−33, M−36 and M−51 correspond to the elimination of CH_3, H_2O, CH_3+H_2O, $2H_2O$ and CH_3+2H_2O respectively.

Fraction no.	*Proposed formula*	*Mol. wt.*	*Mass spectral peaks observed at*								
			M−2	*M−15*	*M−18*	*M−33*	*M−36*	*M−51*	*193*	*231*	*247*
68	$C_{21}H_{32}O_3$	332	×	×	×	×			×		×
69	$C_{21}H_{32}O_3$	332	×		×	×			×	×	
77	$C_{21}H_{32}O_4$	348		×	×	×	×	×	×		×
78	$C_{21}H_{30}O_4$	346	×	×	×		×		(×)	×	
79	$C_{21}H_{30}O_4$	346	(×)	×	×	×	×		×	×	
80	$C_{21}H_{30}O_4$	346	(×)	×	×	×	×	×	(×)	(×)	
82	$C_{21}H_{32}O_4$	348		×	×	×	×		×	(×)	

The mass spectra of fractions 68 and 69 showed parent peaks at *m/e* 332 and fragment peaks at 314 (M−18), corresponding to elimination of one molecule of water, typical for alcohols. This indicates the presence of one hydroxyl group in the terpenic moiety. As the aromatic olivetol moiety contains two oxygen atoms, the main constituents of these fractions should have a total of three oxygens, giving the formula $C_{21}H_{32}O_3$. The mass spectra of fractions 77, 78, 79, 80 and 82 showed fragment peaks both at M−18 and M−36, indicating two hydroxyl groups in the terpenic moieties. By analogy, their formulae should be $C_{21}H_{30}O_4$ and $C_{21}H_{32}O_4$, as seen in Table I.

A mass spectrum of fraction 80 is shown in Fig. 5. A comparison with the spec-

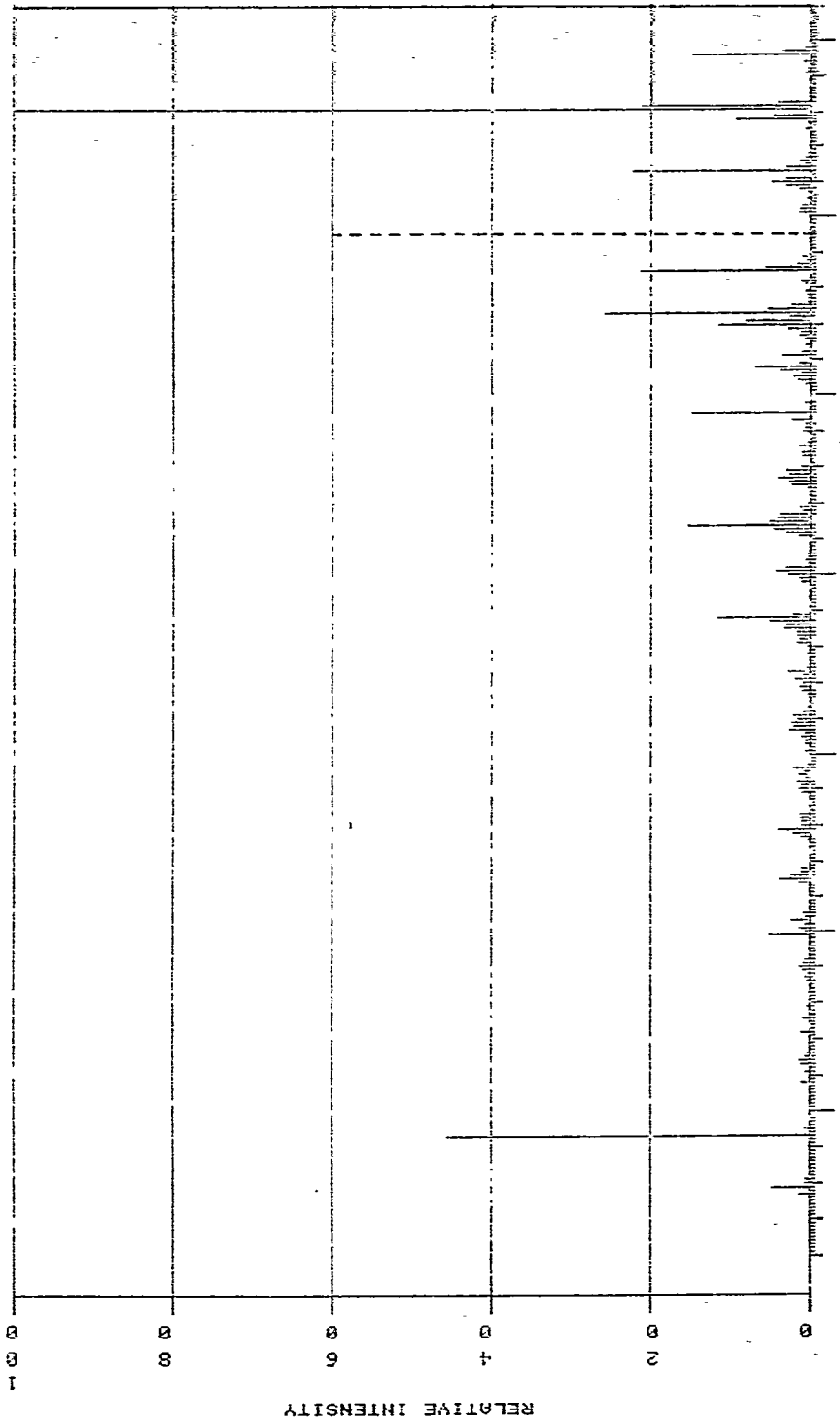

Fig. 5. Mass spectrum of fraction 80. The peak at *m/e* 295 (dashed bar) was not seen in the original spectrum. However, its presence was demonstrated in a later experiment, with an intensity of 60% of the base peak.

trum of $\Delta^{1,2}$-THC obtained under the same conditions indicates that the terpenic moiety of the main constituent of this fraction is fragmented only to a small extent. This may be caused by stabilization of the terpenic moiety by double bonds, originating from the elimination of two molecules of water. The presence of an olivetolic moiety is indicated by the small mass chromatographic peaks at 193 and 231 a.m.u. as seen in Fig. 2. The main constituent of fraction 80 may therefore be $\Delta^{3,4}$-tetrahydrocannabitriol, the CBD carboxylic acid ester of which was reported as a constituent of hashish by Von Spulak *et al.*[10]. The presence of $\Delta^{3,4}$-tetrahydrocannabitriol in cannabis was predicted by Neumeyer and Shagoury[11].

Fig. 6 shows a mass spectrum of fraction 83. It is essentially identical with that of CBN (*cf.* Fig. 7) except that it is shifted by 16 a.m.u. Therefore, it seems likely that fraction 83 is a pure compound constituting a hydroxy derivative of CBN. The lack of an alcoholic fragmentation pattern indicates that the hydroxyl group is situated in one of the aromatic rings. A mass spectrum of fraction 84 showed major peaks at *m/e* 193, 231 and 247, indicating the presence of a cannabinoid. Peaks at higher values including *m/e* 431 were also observed, but the parent peak could not be definitely identified.

As mentioned above, the construction of mass chromatograms was used in the interpretation of some complex mass spectra, resulting from the scanning of mixed GC fractions. Since these mass chromatograms covered the entire retention-time range, some additional minor components were detected, *viz.* 56–59, 61, 66, 70–76 and 81 (*cf.* Fig. 1). These components could not be studied further during access to the instrument. However, it seems likely that components 57 and 59, having relatively short retention times and showing mass chromatographic peaks at *m/e* 328 and 330, respectively, are cannabinoid methyl ethers. Such components have been found in cannabis by Yamauchi and other workers (literature cited in ref. 6). The occurrence of component 59 shows that minor components may be eluted together with the main constituents, such as $\Delta^{1,2}$-THC in this case. This should be remembered when precise GC determinations are to be carried out.

Gas chromatographic retention times

The relative retention times on the OV-17 column of some of the minor components studied are listed in Table II. The effect of the introduction of an additional hydroxyl group on the retention times of cannabinoids may be estimated from retention data of cannabinoid metabolites. Findings of Fonseka *et al.*[12] and experiments made at this laboratory indicate that the introduction of a hydroxyl group into the 6-position of $\Delta^{1,2}$-THC increases its retention time by a factor of *ca.* 2. This supports the conclusions from the MS study that the main constituents of fractions 68 and 69 (*cf.* Table II) are hydroxy derivatives of olivetol cannabinoids, the additional hydroxyl group being located in the non-aromatic moieties. The constituents having molecular weights of 346 and 348, corresponding to two additional hydroxyl groups, show a further approximately two-fold increase in the retention time.

As mentioned above, the MS study indicated that the additional hydroxyl group of component 83 is bonded to aromatic carbon. As seen from Table II, the retention time of this component was *ca.* 4 times larger than that of CBN, due to the introduction of one phenolic hydroxyl group. This increase may seem insufficient, since, for example, the retention time of thymol was *ca.* 8 times longer than that of the

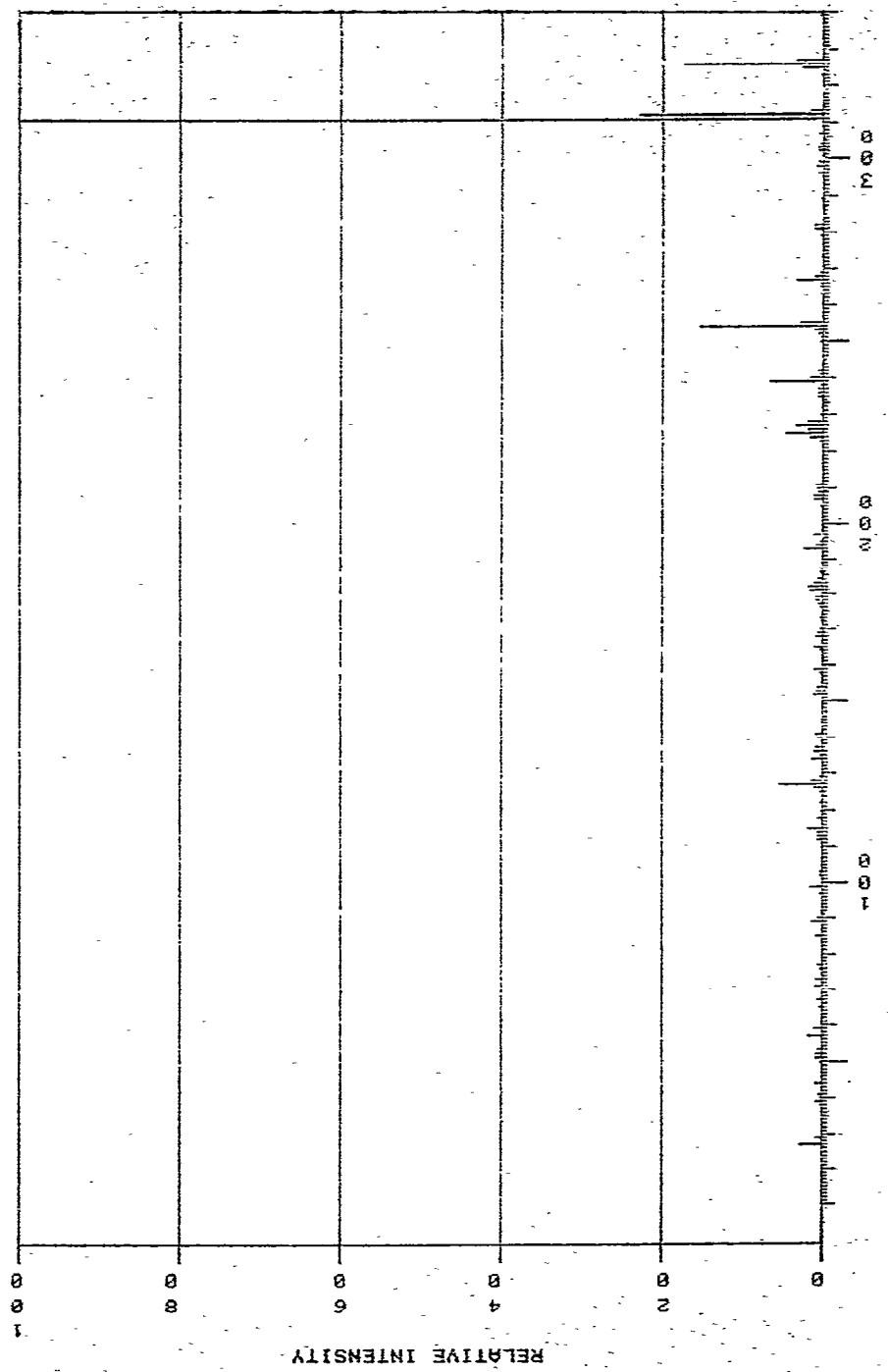

Fig. 6. Mass spectrum of fraction 83.

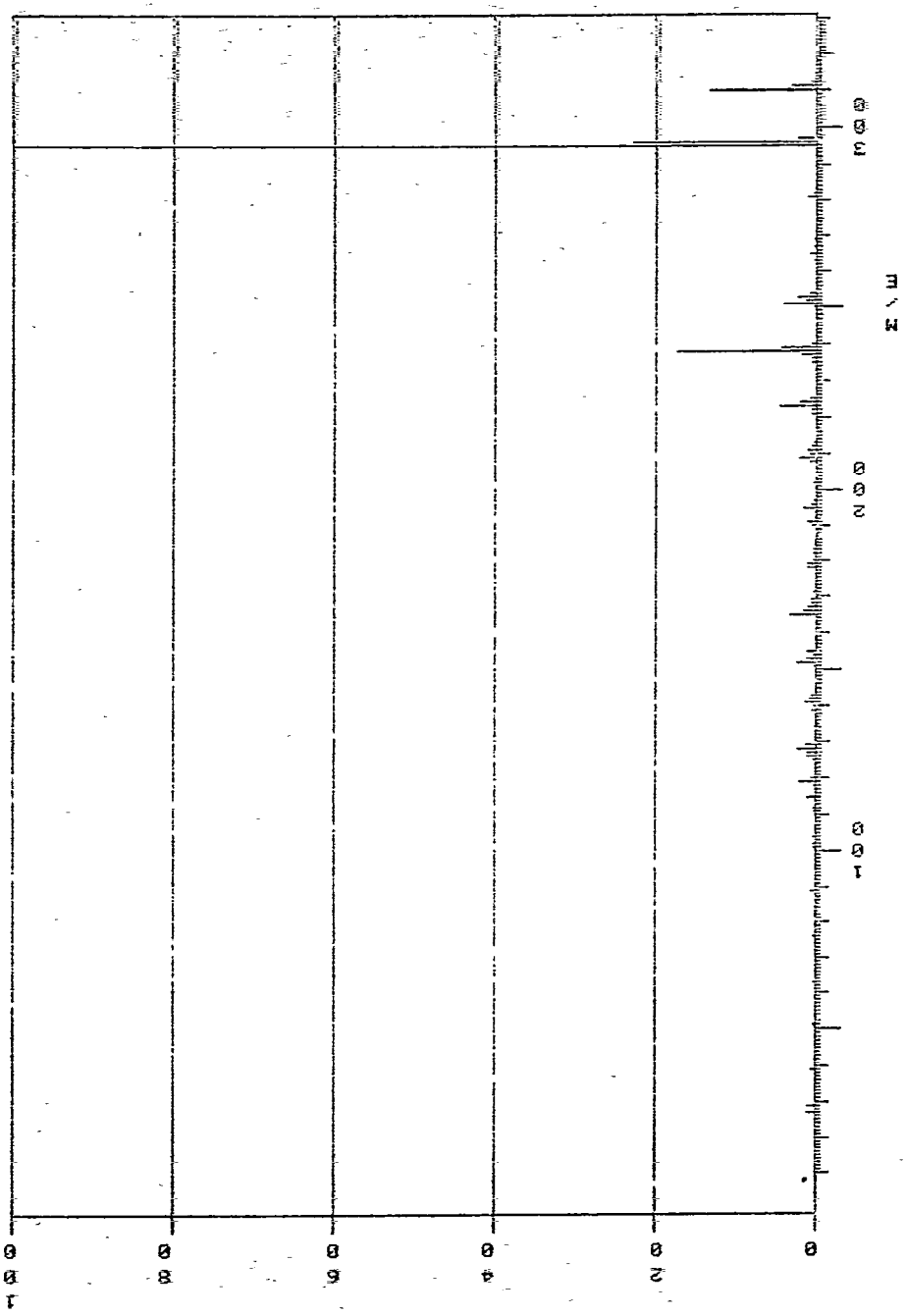

Fig. 7. Mass spectrum of CBN (fraction 63).

TABLE II

RELATIVE RETENTION TIMES OF SOME OF THE GC FRACTIONS STUDIED

Fraction	*Mol. wt.*	*Relative retention time*	*Fraction*	*Mol. wt.*	*Relative retention time*
CBD	314	0.69	78	346	3.04
$\Delta^{1,2}$-THC	314	1.00	79	346	3.39
CBN	310	1.31	80	346	3.76
67	310	1.63	82	348	4.1
68	332	1.65	83	326	4.9
69	332	1.84	84	—	5.6
77	348	2.64			

corresponding hydrocarbon *p*-cymene on OV-17. However, this anomaly may be explained by the known low polarity of cannabinoid phenolic groups.

CONCLUSION

Among the minor components of cannabis resin eluted after CBN, there occur mono- and dihydroxy derivatives of cannabinoids derived from olivetol such as CBD, CBG and CBN. For a closer study of these minor components, a high-resolution chromatographic system is needed such as capillary GC. Silylation may then be necessary to shorten the retention times. High-pressure liquid chromatography may be an alternative. In both cases, the main constituents should be removed prior to analysis in order to avoid overloading of the columns.

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