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## IDENTIFICATION OF CANNABIVARINS IN HASHISH BY A NEW METHOD OF COMBINED GAS CHROMATOGRAPHY-MASS-SPECTROMETRY

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### SUMMARY

The cannabis constituents cannabidiol, tetrahydrocannabinol and cannabinol in hashish are accompanied by the propyl analogues cannabidivarin, tetrahydrocannabivarin and cannabivarin. A new method was developed to identify these compounds by means of the combined gas chromatograph-mass spectrometer.

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### INTRODUCTION

The gaschromatographic analysis of Cannabis constituents has been studied intensively<sup>1</sup>. The compounds are separated by columns packed with 1-5% Se 30, Se 52, Xe 60, Carbowax 20 M and OV 17. The temperatures used are normally very high, 230-250°. In general, two large peaks and a relatively small one can be distinguished at the gaschromatogram. The three peaks are eluted in the following sequence: (1) cannabidiol (CBD), (2) tetrahydrocannabinol (THC) and (3) the smallest peak, cannabinol (CBN).

In the literature little attention is given to the great number of small peaks which are eluted from the column before the cannabidiol (CBD) elutes. This lack of attention is due to the great difficulty encountered in attempting to identify materials that are present in very small concentrations. Preparative gas chromatography or column chromatography, therefore, do not offer a clear solution to this problem. Thin layer chromatography of cannabis has also been studied intensively<sup>2</sup>. With TLC the unknown compounds can be eluted from the plate and brought directly into the mass spectrometer. With the high resolution mass spectrometer, the molecular weight of the compound can be determined with high accuracy. However, using this technique, one has to deal with uncertainty concerning the purity of the compound. When a mixture of compounds have the same R<sub>f</sub> value and this mixture is eluted

and brought into the mass spectrometer, the molecular weight of the largest compound is determined. The accuracy of the method is limited by the relative concentration of the compounds in the sample and the separation technique.

The best technique to date is the combination of gas chromatography with mass spectrometry in one apparatus. This technique gives more evidence about the purity of the compound and can be used for the determination of small amounts of compound. Purity can be checked by using different columns, which have previously been investigated as to their ability to elute all constituents.

Presently we would like to introduce a new method for identification of unknown compounds by means of the mentioned combination GC-MS.

In general, there are no rules for monitoring mass spectra.

Although the temperature of the ion source is often varied during mass spectral analysis, the electron energy utilized is usually a standard of 70 eV. The temperature of the ion source is very important when the compounds are not very stable. For instance, the mass spectrum of  $\Delta^1$ -2 tetrahydrocannabinol (THC) taken with 70 eV at 250° differs markedly from that taken with 70 eV at 100°. With the combination of an electron energy of 70 eV and ion source temperature of 250°, it was impossible to get a mass spectrum that could be used for the identification of compounds with the same molecular weight. The fragmentation pattern is too similar to get any real information about differences in molecular structure. The spectra published in the literature<sup>1,2,3</sup> contain mass fragments with characteristic line intensities (abundance) which can be used for the identification of one pure compound.

However, when, for example, the mass spectrum of THC is taken at 20 eV, 250° and compared to the mass spectrum taken at 70 eV, 100°, it turns out that the line intensities of the fragments 231, 243, 258, 271, 299 and 314 are very characteristic for the compound. This, therefore suggests a possible method for identification which is more specific than the mass spectrum at only one electron energy value.

In our study of the cannabis constituents of natural hashish the temperature of the ion source (250°) of the combined LKB 9000 gas chromatograph-mass spectrometer must be higher than the temperature of the separator (220°) and the temperature of the column (190°) in order to avoid condensation of column material and compound in the ion source. From these data it was established that the combination of the electron energy and the ion source temperature is very important for the mass spectrum of the labile cannabis constituents. In the experimental part and in the results it is outlined how this phenomenon is used for the identification of the cannabis constituents bearing a propyl chain in stead of a pentyl chain.

## EXPERIMENTS

### *Gas chromatography*

A Hewlett Packard 402 gas chromatograph with flame ionisation detector was used. The temperature of the oven was 190°, the temperature of the injection block 220° and the temperature of the detector 220°. The nitrogen flow was 40 ml/min, hydrogen flow 40 ml/min and air flow 200 ml/min. The column was packed with 3% OV 17 at Diatoport S 60-80 m 1.80 m, diameter 3 mm.

*Gas chromatography-mass spectrometry*

An LKB 9000 gaschromatograph-mass spectrometer was used. The column was packed with 3% OV 17 on Diatoport S-60-80 m. The length of the column was 1.80 m and the diameter 3 mm.

The temperature of the oven was 180°, the temperature of the separator 220° and the temperature of the ion source was 250°.

The electron energy was varied from 10 eV to 20 eV in order to construct the energy-mass intensity graphs. The trap current was maintained at 60  $\mu$ A. The helium flow was 30 ml/min. The gas chromatogram of the hashish samples separated isothermally at 180° were identical with the gaschromatograms obtained with a temperature program of 5 min 150° and temperature increase of 2°/min up to 220°.

Mass spectra were taken at the increase slope of the gaschromatographic peak. The electron voltage indicator of the instrument was calibrated at the ionisation energy of helium, 24.4 eV.

The mass spectra were normalized with the base peak equal to 100% intensity and graphs of line intensity of a particular mass fragment at a particular electron volt were constructed.

*Sample preparation*

The hashish was powdered and extracted with *n*-hexane. The hexane extract was concentrated to 0.5 ml in order to get a concentrated solution of compounds which are normally present in small concentrations. 8  $\mu$ l was injected into the LKB 9000 gas chromatograph-mass spectrometer.

## RESULTS AND DISCUSSION

Tetrahydrocannabinol (THC) may exist in several geometrical isomers (double bond position and cis-trans isomers), but two forms seem to occur in nature. These isomers are called: trans  $\Delta^1$ -2 THC and trans  $\Delta^1$ -6 THC (Fig. 1). Pure  $\Delta^1$ -2 THC and  $\Delta^1$ -6 THC show a fragmentation pattern in the mass spectrometer as illustrated in Fig. 2. Both compounds give at 20 eV, 250°, the same mass fragments but with large differences in the line intensities of these various mass-fragments.

With the energy fixed at 20 eV, 250°, the fragmentation pattern may be compared with a reaction sequence, in which the line intensities of the various mass fragments are a measure for the concentration of the ions in the reaction mixture.

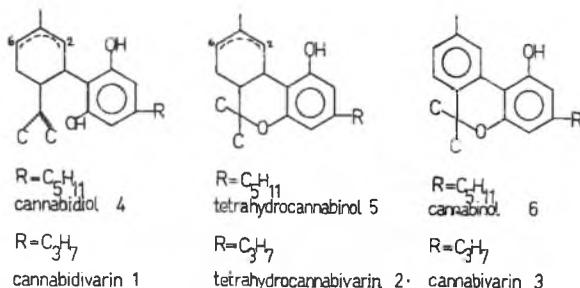
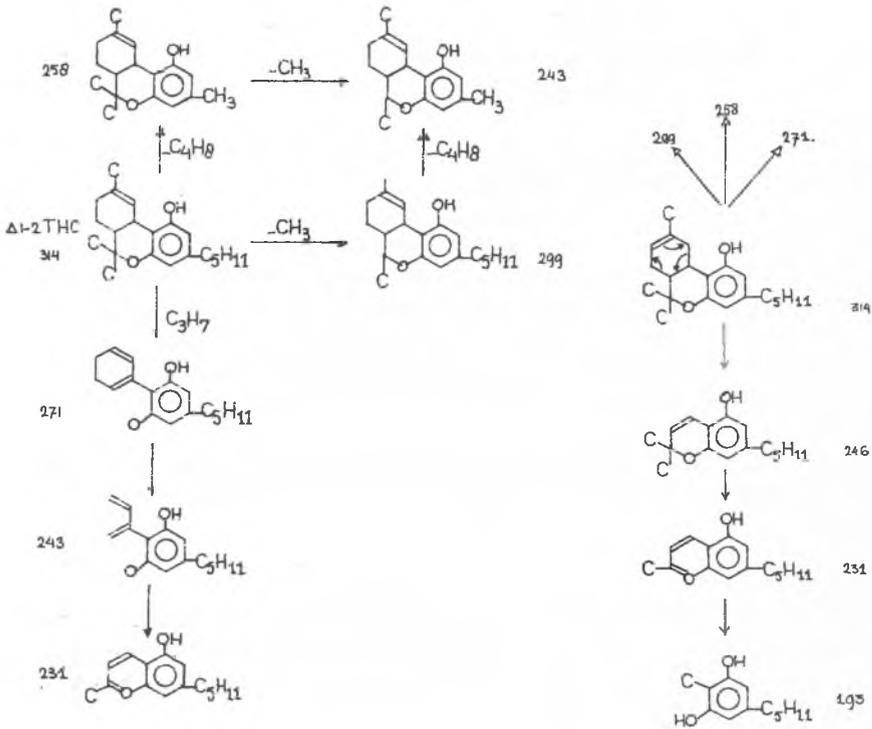


Fig. 1. Molecular structure of cannabidiol, tetrahydrocannabivarin, cannabivarin, tetrahydrocannabinol and cannabivarin.



fragmentation pattern of  $\Delta^1$ -2 THC      fragmentation pattern of  $\Delta^1$ -6 TH  
 Fig. 2. Fragmentation pattern of  $\Delta^1$  1-2 THC and  $\Delta^1$  1-6 THC according to Budzikiewicz<sup>1</sup>.

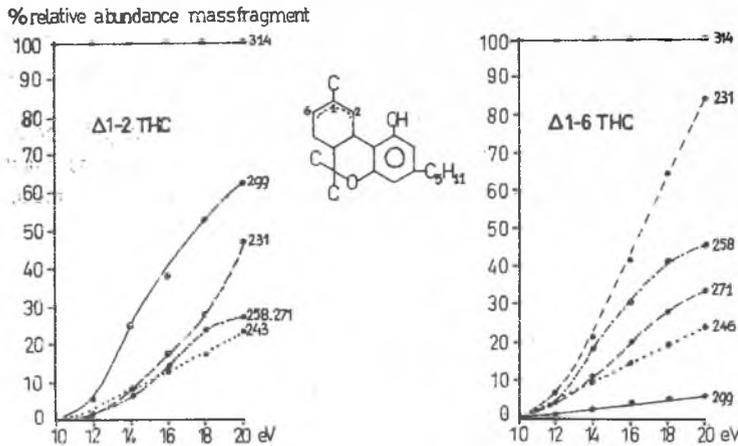


Fig. 3. Electron-voltage-massfragment intensity graphs of  $\Delta^1$  1-2 THC and  $\Delta^1$  1-6 THC. The  $\Delta^1$  1-6 THC is converted much faster into the mass fragment 231 than the  $\Delta^1$  1-2 THC.

The combination electronenergy—ionsource temperature is an energy content, which may determine the rate of the reaction. If the energy content is altered the reaction may occur faster or slower. With this assumption the mass spectrum of  $\Delta 1-2$  and  $\Delta 1-6$  THC is taken at 10, 12, 14, 16, 18, and 20 eV, 250° C and normalized (largest peak = 100%). The percentage of the line intensities, which are a measure for the concentration of the ions are plotted against the energy eV, 250°.

Fig. 3 shows the differences in the reaction rate of mass 314 to mass 231 between  $\Delta 1-2$  THC and  $\Delta 1-6$  THC. The mass fragment 231, which both compounds have in common (Fig. 2), may be considered as the terminal in the reaction sequence. The  $\Delta 1-6$  THC has been converted faster into the mass fragment 231 than the corresponding  $\Delta 1-2$  THC. This means that less energy is required for the degradation of  $\Delta 1-6$  THC than for  $\Delta 1-2$  THC. So the  $\Delta 1-2$  isomer has a more rigid structure than the  $\Delta 1-6$  isomer. The graphs eV-percentage occurrence of a mass fragment can be used for the identification of the compounds. The mass spectra of both THC molecules show from 10–20 eV the molecular peak (314) as the base peak (100%). This observation is very important, because the cannabidiols, having the same molecular weight of 314, show a similar mass spectrum with similar mass fragments, but the line intensities differ completely. The molecular peak of 314 decreases rapidly from 100% at 12 eV to 10% at 20 eV, but at the same time the mass fragment 246 increases to the maximum 100% at 14 eV and subsequently decreases. The mass fragment 231 becomes at 13 eV the most important one (100%) (Fig. 4.4). Mass fragment 321 has reached a 50% line intensity at 13 eV,  $\Delta 1-6$  THC at 16,5 eV and  $\Delta 1-2$  THC at 20 eV. This means that the energy of activation in the reaction of mass fragment 314 to 231

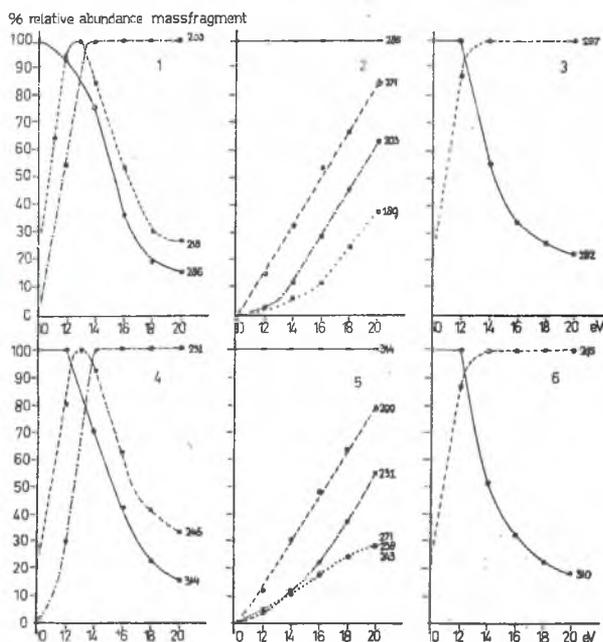


Fig. 4. Electronvoltage—massfragment intensity graphs of cannabivarins (4.1)  $\Delta 1-2$  tetrahydrocannabinol (4.2) cannabivarins (4.3) cannabidiol (4.4)  $\Delta 1-2$  tetrahydrocannabinol (4.5) and cannabiol (4.6).

is in cannabidiol (CBD) 7 eV less than with  $\Delta^1$ -2 THC. The most characteristic feature in this fragmentation pattern of CBD is the rise and fall of mass 246, which has its maximum at 13 eV.

In nature, only  $\Delta^1$ -2 CBD seems to be a stable compound and it occurs in hashish samples from all origins. The intensities of the masses 314, 246 and 231 of cannabidiol taken at various energies are very characteristic and can be used for the identification of the compound.

In a similar way cannabinol (CBN) shows a very distinctive energy-mass intensity graph. The CBN has a structure, that, due to the two aromatic rings, is more rigid than the THC and CBD molecules. The mass spectrum of CBN shows two masses with relative high intensities: the molecular peak of 310 and a mass fragment 295, due to the loss of a geminal methyl group. The mass fragment 231 is not formed at all. Fig. 4.6 shows the behaviour of the intensities of the mass fragments when the energy of the mass spectrum is varied. With this technique the three major components in natural hashish can easily be distinguished. From these figures it can be deduced that with the above mentioned compounds, the fragmentation of the alicyclic ring systems, rather than the fragmentation of the  $C_6$  chain, is the predominant route of fragmentation. If the fragmentation of the  $C_6$  chain was the major pathway, the CBD, THC and CBN would show a similar spectrum and a similar energy-mass intensity graph.

When a gaschromatogram of a hashish extract is made at a 3% OV 17 column with a fairly low temperature of 180°, then more peaks can be distinguished than the three mentioned in the literature. In particular, the compounds which are eluted before the known peaks of CBD, THC and CBN are of special interest. In general, the concentrations of these unknown compounds in the hashish are very low and little attention has been paid to these compounds. The apparatus required is the combined gaschromatograph-mass spectrometer, in order to construct fragmentation patterns at different eV as quick as possible from the small peaks. The same difficulty arises as with the TLC separation, namely the question of the purity of the samples. When a gaschromatographic peak is a mixture of geometric isomers, a fragmentation pattern is obtained of all the compounds. However, the structure of the compounds in a mixed gaschromatographic peak can be elucidated when the fragmentation patterns of some compounds are known.

In general 10–20 compounds elute from the 3% OV 17 column before the CBD elutes. The concentrations of these compounds vary with the origin of hashish. Now in the gaschromatogram of an extract of Nepalese hashish, three distinct peaks can be distinguished with a shorter retention time than that of CBD (Fig. 5). In this Nepalese sample the concentrations of these three compounds are relatively high, compared with the same compounds occurring in Maroc, Turkish and Lebanese hashish.

When the energy-mass intensity graphs are constructed of these three compounds as done with the CBD, THC and CBN, it is seen that there is a very great analogy with these compounds. The molecular weight of the three compounds is 286, 286 and 282. This means a constant factor 28 less than the molecular weights 314, 314 and 310. From the graphs in Fig. 4 it can be seen that every mass fragment mentioned can be converted into a corresponding mass fragment of the known structures CBD, THC and CBN, by adding mass 28.

The shape of the curves of the energy-mass intensity are the same. The mass

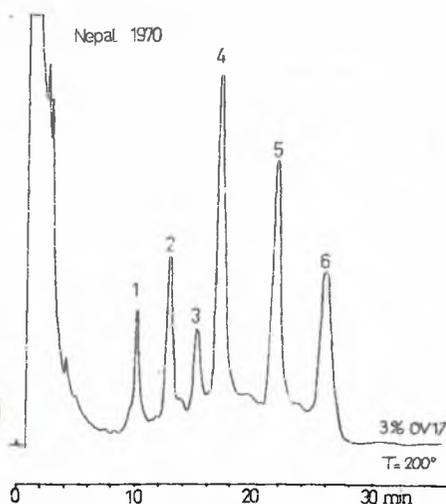


Fig. 5. Gaschromatogram of an extract of Nepalese hashish made on a column packed with 3% OV at 200°. Compound 1 is cannabidivarin, compound 2,  $\Delta^1$ -2 tetrahydrocannabivarin, compound 3, cannabivarin, compound 4, cannabidiol, compound 5,  $\Delta^1$ -2 tetrahydrocannabinol and compound 6, cannabinal.

fragment 231 reaches 50% of the relative intensity for the various compounds at the given electron volts (see Fig. 4.4; 4.5) cannabidiol 13 eV,  $\Delta^1$ -2 THC 20 eV.

In the Nepalese hashish the compounds with the molecular weight of 286 are converted into mass fragment 203 (231-28). The mass fragment 203 reaches 50% of the relative intensity for the various compounds at the given eV: (see Fig. 4.1 and 4.2) compound 1 at 13 eV, compound 2 at 20 eV and with compound 3 no mass fragment 203 is formed. This means that the fragmentation occurs in the common part of the molecules and as we know that fragmentation takes place in the alicyclic ring system, this part of the molecule should be the same. Compound 1 corresponds with compound 4 (CBD), 2 with 5 (THC) and 3 with 6 (CBN) see Fig. 4 and Fig. 5. This is the absolute proof that the CBD, THC and CBN are accompanied by the corresponding compounds bearing a propyl chain instead of a pentyl chain.

The compounds eluted from the column are: (1) cannabidivarin, (2)  $\Delta^1$ -2 tetrahydrocannabivarin and (3) cannabivarin (Fig. 5). Also in other hashish samples evidence was found for the occurrence of cannabidivarin by Volner<sup>6</sup>. Tetrahydrocannabivarin was identified by Gill<sup>5</sup> as a constituent of cannabis tincture. Merkus<sup>7</sup> suggested the existence of cannabivarin. The compound was eluted from a thin layer plate and brought directly into the mass spectrometer. No information concerning the purity was given. Similar experiments in our laboratory show that the eluted compound from the plate shows 5-10 peaks in the gaschromatogram at 3% OV 17 at 180°. The mass spectra of these peaks show that the compounds were cannabis constituents. Thus the mass spectrum of an eluted compound alone does not give enough information about the structure of the compound, when data about the purity are omitted. When it is impossible to obtain IR and NMR spectra of the compound, the fragmentation pattern of the compound at various energies gives further information.

The existence of all three propyl analogues in one hashish sample has never

been published. In fact in all hashish samples we examined the three propyl compounds are present. There seems also to be a definite correlation between the concentration of the pentyl and the propyl analogue present in one sample. From the biogenetic point of view this is an interesting feature. Further investigation is necessary to elucidate exact correlations in a great number of hashish samples from different origin.

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