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**CANNABINODIOL, A NEW HASHISH CONSTITUENT,
IDENTIFIED BY
GASCHROMATOGRAPHY-MASS SPECTROMETRY**

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SUMMARY:

In extracts of Nepalese hashish and Brazilian marihuana a hitherto unknown cannabinoid component was found. On the basis of its behaviour in gaschromatography and mass-spectrometry and in comparison with a number of cannabinol like substances, the aromatic cannabidiol, with the trivial name 'cannabinodiol' is proposed as the most probable structure for the new compound.

I. INTRODUCTION.

Although much attention has been paid to the analysis and identification of the constituents of Cannabis preparations like hashish and marihuana (see for reviews: Mechoulam(1970), Neumeyer and Shagoury(1971)), the use of new techniques of combined gaschromatography-mass spectrometry recently enabled us to demonstrate the occurrence in hashish and marihuana of several (until then unknown) analogues of the major cannabinoid constituents (Vree, Breimer, Van Ginneken, Van Rossum, De Zeeuw and Witte, 1971; Vree, Breimer, Van Ginneken and Van Rossum, 1972; Breimer, Vree, Van Ginneken and Van Rossum, 1972; De Zeeuw, Wijsbeek, Breimer, Vree, Van Ginneken and Van Rossum, 1972).

The method consists in taking mass-spectra at various electron energies, ranging from 20 to 10 electronvolt (eV), and plotting the relative intensities of particular massfragments versus the electron energies used. Characteristic graphs are obtained in that way for each cannabinoid (electronvoltage-massfragment intensity graphs).

The fragmentation behaviour of the cannabinoids appears to be mainly determined by the alicyclic ringsystem (Budzikiewicz, Alpin, Lightner, Djerassi, Mechoulam and Gaoni, 1965; Claussen, Fehlhäber and Korte, 1966).

Variations in the length of the side chains of the aromatic ring on the other hand do not have important consequences for the fragmentation pattern in the electron-voltage range we use (10-20 eV), but they seem to be crucial for the gaschromatographic behaviour of the various cannabinoids (unpublished observations in our laboratory).

Another aspect is the rate of fragmentation of a molecular ion into certain massfragments. It is reasonable to assume that differences in the electron energy, at which the same massfragment originates from different molecular ions, reflect in some way the stability of the parent compounds. Actually the term 'stability' is used here from a kinetic point of view, so the differences mentioned refer to the activation energy of a certain fragmentation, and not to a strictly thermodynamic property of the parent compound.

Comparing for instance the electronvoltage-massfragment intensity graphs for cannabicyclol, cannabichromene, cannabidiol, Δ^{1-6} tetrahydrocannabinol and Δ^{1-2} tetrahydrocannabinol, which have the same molecular weight (314) and which have the same fragment of mass 231 in common, we find that massfragment 231 reaches a relative intensity of 50 % at 10, 12, 13, 16, and 20 eV respectively.

This means that the activation energy for the different mechanisms of fragmentation from molecular mass 314 to massfragment 231 is increasing in the order: cannabicyclol, cannabichromene, cannabidiol, Δ^{1-6} tetrahydrocannabinol and Δ^{1-2} tetrahydrocannabinol. These findings afford some interesting features: While the relative instability of cannabicyclol and cannabichromene is hardly surprising on the basis of molecular structure, it is suggested that fragmentation of cannabidiol to massfragment 231 does not proceed via tetrahydrocannabinol. The difference between the two tetrahydrocannabinols is fully explicable by the fact that unlike the Δ^{1-2} isomer, Δ^{1-6} tetrahydrocannabinol is subject to a rather easily occurring retro Diels Alder reaction (Budzikiewicz, Alpin, Lightner, Djerassi, Mechoulam and Gaoni, 1965; Claussen, Fehlhauer and Korte, 1966).

It should be emphasized that we are dealing with overall activation energies, so we can not get any information about the complexity of the fragmentation and the relative importance of the various processes involved. Comparison of the several fragmentation reactions, structures and energies may give an answer to the differences in internal energies of the molecules.

Obviously comparisons are much less complicated, as far as simple unambiguous transitions are concerned.

II MATERIALS AND METHODS

A. Sample preparation

Nepalese hashish and Brazilian marihuana were powdered and extracted with n-hexane. After filtration, part of the solvent was evaporated in order to obtain suitable concentrations for gaschromatography and mass-spectrometry.

B. Gaschromatography

A Hewlett-Packard 402 gaschromatograph with flame-ionisation detector was used. Glass columns, 1.5 m, inner diameter 3 mm, were packed with 3 % UCW98 on Gas Chrom Q 60-80 mesh. Temperature of oven 180°C, injection block 230°C, detector 230°C. Nitrogen flow-rate 30 ml/min; hydrogen flow 30 ml/min; air flow 150 ml/min.

C. Gaschromatography-mass spectrometry

An LKB 9000 combined gaschromatograph-mass spectrometer was used. Same columns used as described under Gaschromatography. Helium flow 30 ml/min. The oven temperature was 180°C, temperature of the separator 220°C and temperature of the ion source 250°C.

The trap current was maintained at 60 μ A, the accelerating voltage at 3.5 kV. Repetitive mass spectra were taken at different electron energies between 20 and 10 eV during the elution of a component from the gaschromatograph, as recorded by the total ion current at 20 eV.

Mass spectra were normalized (base peak = 100 %) and electron-voltage-massfragment intensity graphs were constructed

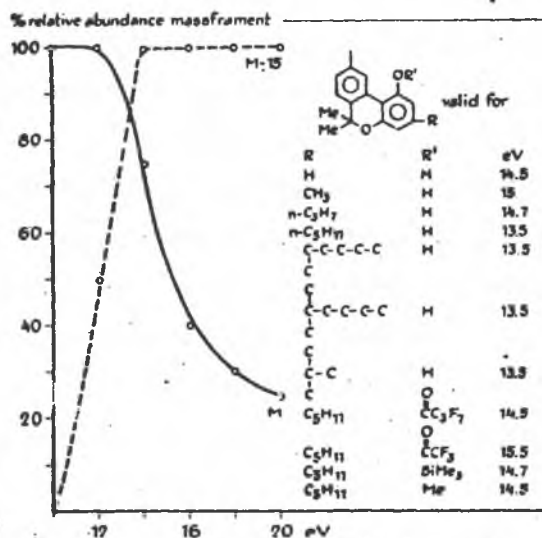


Figure 1.

Electron voltage-massfragment intensity graphs for a number of cannabinol like compounds. The 'eV' refers to the crossing point of the intensity lines for the molecular ion (M) the massfragment M-15.

III. RESULTS AND DISCUSSION

In: the normal, wellknown cannabis constituent cannibinol (see figure 1, $R = nC_5H_{11}, R' = H$) fragmentation is at the electron energies used practically restricted to the loss of a geminal methyl group, so the compound shows a highly 'characteristic' mass spectrum : molecular ion $M=310$, massfragment $M-15 = 295$ and a metastable peak at mass 281. Only one further massfragment is observed, mass 254, which originates from the loss of a C_4H_8 fragment in the pentyl side chain. (Stepwise fragmentation of the side chain does practically not occur. This holds true for all known natural cannabinoids and from unpublished experiments it was found that also for olivetol (4-n-pentyl-resorcinol) stepwise fragmentation of the pentyl side chain accounts for only 1-6% of the total fragmentation). However this pathway is of minor importance at the electron energies used and accounts for a few percent maximally. When the relative intensities of the masses 310 and 295 were plotted versus the electron energies used, a typical graph was obtained (figure 1), the 'crossing point' of the lines for mass 310 and 295 being at about 14 eV. This crossing point reflects in some way the activation energy for the reaction resulting in the loss of a geminal methyl group, as was pointed out in the introduction. Totally analogous graphs are obtained for the cannabinol like compounds, in which the pentyl side chain is replaced by a propyl or methyl side chain. (Vree, Breimer, Van Ginneken, and Van Rossum, 1972; Vree, Breimer, Van Ginneken, Van Rossum, De Zeeuw and Witte, 1971).

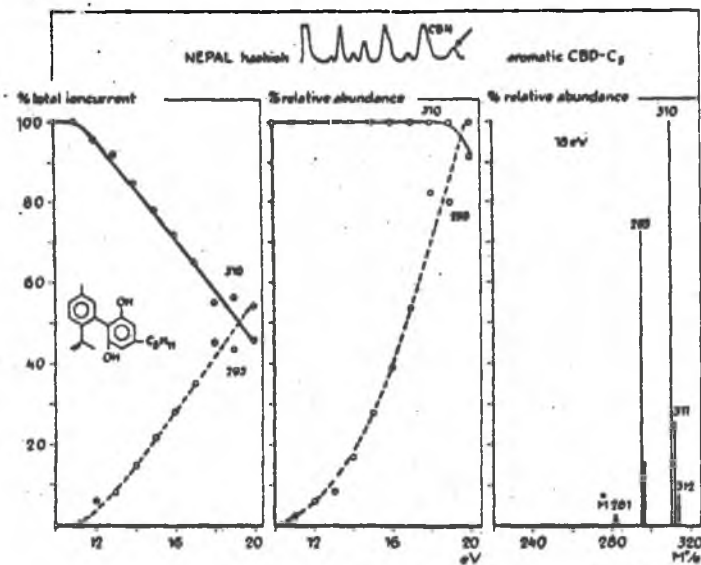


Figure 2.

Total ion current diagram for an extract of Nepalese hashish and the electronvoltage-massfragment intensity graph for the new compound, which elutes just after cannabinol.

The depicted structure (aromatic cannabidiol or cannabinodiol) is explained in the text.

The observation that no fragmentation of the ringsystem takes place is interesting in view of the difference in mass spectrometric behaviour of Δ^{1-2} and Δ^{1-6} tetrahydrocannabinol. In contrast to its Δ^{1-6} -isomer, Δ^{1-2} THC shows a relatively high intensity for massfragment 299 (Vree, Breimer, Van Ginneken, Van Rossum, De Zeeuw and Witte, 1971). This is caused by the fact that in Δ^{1-6} THC fragmentation of the ringsystem is predominant.

So when only the mass fragments of the molecular ion M and M-15 are observed in the mass spectra, this has to be explained by a high stability of the ringsystem of the molecule.

Another important finding is that the cannabinol like mass spectrum is largely independent of the substituents R and R' (figure 1). The shape of the electronvoltage-mass fragment intensity graphs remains the same and only minor shifts in the crossing point between the M and M-15 lines appear (14 eV \pm 1 eV). When studying extracts of Brazilian marihuana and Nepalese hashish we found in the gaschromatogram an unknown peak eluting just after cannabinol (figure 2), which at first sight had the same mass spectrum as cannabinol : mol ion 310 and mass fragments 295, 254 and a metastable peak at mass 281. In contrast with cannabinol, the crossing point of the lines M and M-15 was at 19.5 eV, about 5 eV higher than for cannabinol.

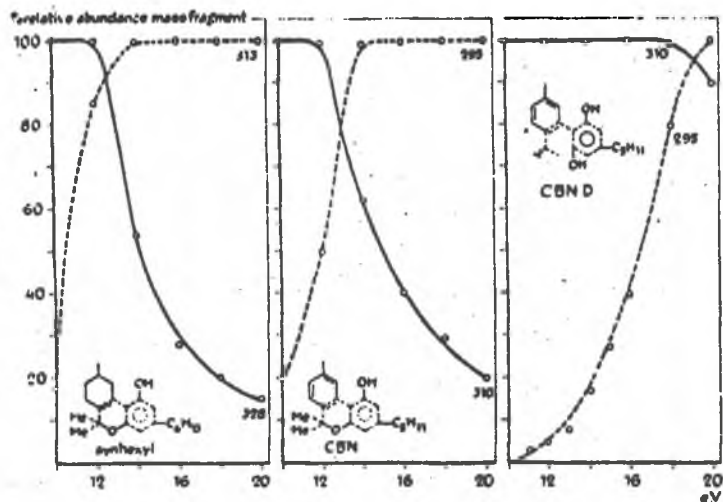


Figure 3.

Electronvoltage-massfragmentintensity graphs for synhexyl, cannabinol (CBN) and cannabinodiol (CBND). See text for further explanation.

From the gaschromatographic and mass-spectrometric behaviour we conclude that the new compound should have a cannabinoid-structure. The molecular weight of 310 indicates that the compound has 4 H atoms less than tetrahydrocannabinol, cannabidiol, cannabicyclol and cannabichromene.

The possibility that the pentyl side chain has lost 4 H atoms can be ruled out, since variations in the side chain do not change the mass-spectrum of the cannabinoids into a cannabinol like mass-spectrum.

The difference of 5 eV between the crossing-points for the M and M-15 lines of cannabinol and the new compound must be due to an increase in conjugation of the ringsystem, leading to more possible resonance structures and thus to a higher stability of the new molecule.

The observation that also synhexyl (see figure 3) shows a cannabinol like mass-spectrum allows us to define more exactly the structural requirements for cannabinoids to show this particular mass-spectrometric behaviour.

It must be concluded that the presence of a double bond in the 3-4 position in conjugation with the aryl-nucleus endows the molecule with a so much increased stability that fragmentation is restricted to the loss of one geminal methylgroup.

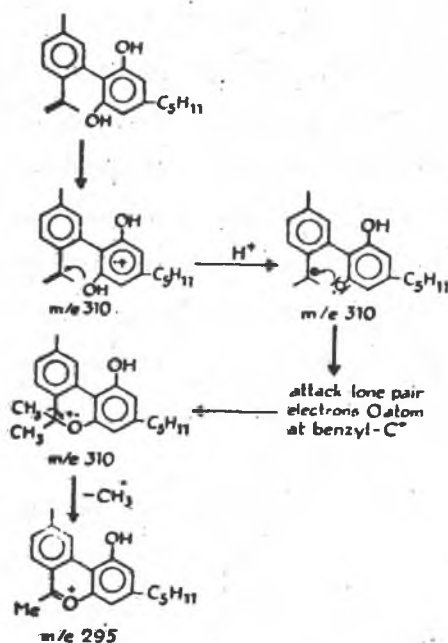


Figure 4.

The proposed fragmentation pattern of cannabinodiol.

The fact that the crossing point of the M and M-15 intensity lines is at about the same electron-energy for syn-hexyl as for the cannabinoids, suggests that further conjugation of the cyclohexene-ring does not give a shift in the crossing points. So the new cannabis constituent should show a still more extensively conjugated structure.

A structure which will fulfill all the requirements mentioned above is the aromatic cannabinodiol, for which we would propose the trivial name cannabinodiol (CBND in figure 3). It is clear from the structures depicted in figure 3, that this compound has still more resonance structures than cannabinol and that in all respects this is the most logical solution to the problem.

Concerning the fragmentation of cannabinodiol, it seems hardly plausible, that the molecule would lose a methyl group directly, so we assume that after a primary ionisation, a cyclisation will occur to a cannabinol-ion (mass 310), which in turn will lose a geminal methyl group leading to the stable massfragment 295. This proposed fragmentation pattern is depicted in figure 4.

From our previous work we have evidence that each cannabinoid bearing a pentyl sidechain is accompanied by propyl- and methyl-analogues (Vree, Breimer, Van Ginneken and Van Rossum, 1972).

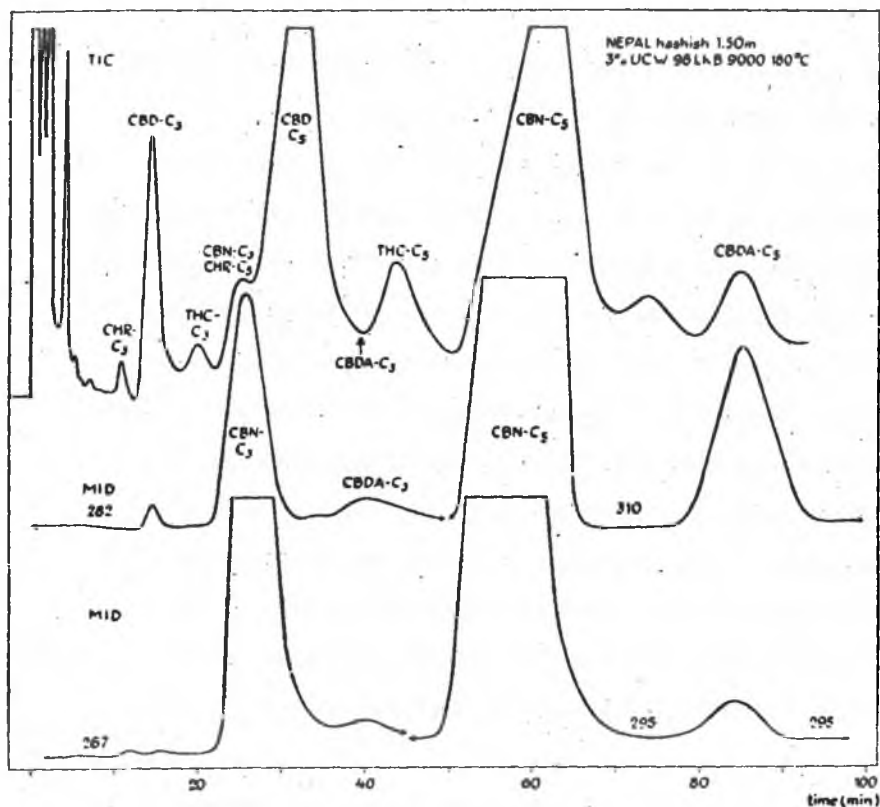


Figure 5.

Total ion current diagram (TIC) and massfragment intensity diagrams (MID) for an extract of Nepalese hashish.

CHR= cannabichromeen, CBD= cannabidiol, THC= Δ^12 tetrahydrocannabinol, CBN= cannabinol and CBDA= aromatic cannabidiol-cannabinodiol.

The appendices C_3 and C_5 refer to the number of carbon atoms in the side chain.

See text for further explanation.

The question arised wether the cannabinodiol with a propyl side chain existed and could be detected.

The proof of the existence, but also the relative concentration in the particular hashish extract and the detection-limit were given by means of the method of massfragmentography (see: Hammar, 1971). First the massfragmentogram was made of mass 310, and just after cannabinol the second compound with molecular weight 310 eluted. The same was done for mass 295. Also when massfragmentograms were made of mass 282 and 267 a second but small peak of the same mass eluted just after the known propyl-homologue of cannabinol (see figure 5). The concentration of the propyl-homologue of cannabinodiol was very low and it was impossible to obtain a mass spectrum with our procedure.

With the technique of massfragmentography the retention-time of the cannabinodiol-propyl-homologue was determined. It was observed with the propyl- and pentyl-homologues of cannabidiol, tetrahydrocannabinol and cannabinol, as identified by combined gaschromatography-mass spectrometry, that there was a fixed ratio between the retention-times. The ratio is independent of oven-temperature and carrier-gas flow, but dependent upon the column liquid phase. From figure 6 it can be observed that the ratio of the

Retentions (cm²) of cannabis homologues in Nepalese hashish (LKB 9000; UCW 98 3%; 180°C)

Chromeen	- C3	5.2	1.00
	- C5	12.2	2.35
CBD	- C3	6.9	1.00
	- C5	15.6	2.28
THC	- C3	9.7	1.00
	- C5	21.2	2.19
CBN	- C3	12.2	1.00
	- C5	30.1	2.48
Arom.CBD	- C3	19.7	1.00
	- C5	40.7	2.10

1 cm = 2 min.

Figure 6.

Comparative retentions of propyl- and pentyl-cannabinoids. For abbreviations see figure 5.

See text for further explanation.

retention-times of the pentyl and propyl-cannabinodiol fits well in the range of ratios for the other cannabinoids. From both mass-fragmentography and retention-behaviour one might conclude that cannabinodiol indeed is accompanied by its propyl analogue. The existence of a methyl analogue could not be demonstrated, probably because of a too low concentration.

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REFERENCES

Breimer, D.D.; Vree, T.B.; Van Ginneken, C.A.M. and Van Rossum, J.M. (1972):

Some New Cannabis-Constituents.

In: Biochemical and pharmacological aspects of dependence and Reports on marihuana research. Edited by H.M. van Praag.

De Erven F. Bohn N.V., Haarlem, The Netherlands.

Budzikiewiez, H.; Alpin, R.T.; Lightner, D.A.; Djerassi, C.; Mechoulam, R. and Gaoni, Y. (1965):

Massenspektroskopische Untersuchung der Inhaltsstoffe von Haschisch.

Tetrahedron, 21:1881-1888.

Claussen, U.; Fehlhaber, H.-W. and Korte, F. (1966):

Massenspektrometrische Bestimmung von Haschisch-Inhaltstoffen II.

Tetrahedron, 22:3535-3543.

Hammar, C.G. (1971) :

Massfragmentography and elemental analysis by means of a new combined multiple ion detector-peak matcher device.

Acta Pharmaceutica Suecica, 8: 129-152.

Mechoulam, R. (1970):

Marihuana Chemistry.

Science, 168: 1159-1166.

Neumeyer, J.L. and Shagoury, R.A. (1971):

Chemistry and Pharmacology of Marihuana.

Journal of Pharmaceutical Sciences, 60: 1433-1457.

Vree, T.B.; Breimer, D.D.; Van Ginneken, C.A.M.; Van Rossum,

J.M.; De Zeeuw, R.A. and Witte, A.P. (1971) :

Identification of Cannabivarin in hashish by a new method
of combined Gaschromatography-Mass-spectrometry.

Clinica Chimica Acta, 34: 365-372

Vree, T.B.; Breimer, D.D.; Van Ginneken, C.A.M. and Van
Rossum, J.M. (1972):

Identification in hashish of tetrahydrocannabinol, cannabidiol
and cannabinol analogues with a methyl side-chain.

Journal of Pharmacy and Pharmacology, 24: 7-12

De Zeeuw, R.A.; Wijsbeek, J.; Breimer, D.D.; Vree, T.B.;

Van Ginneken, C.A.M. and Van Rossum, J.M. (1972):

Cannabinoids with a propyl side-chain in Cannabis:
Occurrence and chromatographic behavior.

Science, 175: 778-779.
