J. Pharm. Pharmac., 1972, **24**, 7–12 *Received September* 9, 1971

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

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This work was supported in part by grants from the Netherlands' Organization for the Advancement of Pure Research (Z.W.O.) and from the Ministry of Health.

The cannabis constituents Δ^1-tetrahydrocannabinol, cannabidiol and cannabinol are accompanied by their homologues with an n-propyl and with a methyl side-chain. The identification procedure by combined gas chromatography-mass spectrometry in hashish and marihuana is described and the consequences for cannabis use are discussed.

In recent years much research has been directed towards the qualitative and quantitative analysis of *Cannabis sativa* preparations, such as hashish and marihuana. Several thin-layer chromatography and gas-liquid chromatography systems have been used for the separation of the various constituents (see Mechoulam, 1970).

Most if not all of the naturally occurring neutral cannabinoids are soluble in light petroleum or hexane and these solvents are often used for extraction and sample preparation. In these samples most attention in the separation and identification procedures has been paid to the main hashish constituents: Δ^1-tetrahydrocannabinol (Δ^1-THC)*, cannabidiol (CBD) and cannabinol (CBN). The Δ^1-THC is thought to be the major psychotomimetically active component (Mechoulam, Shamni & others, 1970).

In general the three major cannabinoids are gas chromatographically well separated on stationary phases of the SE or OV type and they elute in the following sequence: CBD, Δ^1-THC, CBN. The retention times cited in the literature are fairly short, mainly because of the high temperature of the column oven: 230°–250°.

Little attention so far has been paid to the small but distinct peaks in the gas chromatogram of hashish and marihuana extracts, which appear when the column temperature is lowered. Most elute before the three main components and some have been identified e.g. cannabichromene, cannabicyclol (Gaoni & Mechoulam, 1971).

Before beginning work on the pharmacokinetics and metabolism of hashish and its constituents, the chemical composition of different samples was examined and a new method of identification was developed by means of varying the electron energy in a combined gas chromatograph-mass spectrometer. At different electron voltages mass spectra of the components eluting from the gas chromatograph were taken and the relative intensity of the main mass fragments was plotted against the eV (Vree, Breimer & others, 1971). In this way typical graphs were obtained and so we were able not only to identify unambiguously the three main components in hashish samples, but also some of the smaller peaks in the gas chromatogram as the homologues of Δ^1-THC, CBD and CBN with an n-propyl side-chain instead of an n-pentyl side-chain (Vree & others, 1971). Separately these propylcannabinoids had been isolated from hashish (Vollner, Bieniek & Korte, 1969; Gill, 1971; Merkus, 1971). Our unpublished finding of the occurrence of both series of homologues in many hashish samples,

* Numbered Δ^9 according to IUPAC rules.

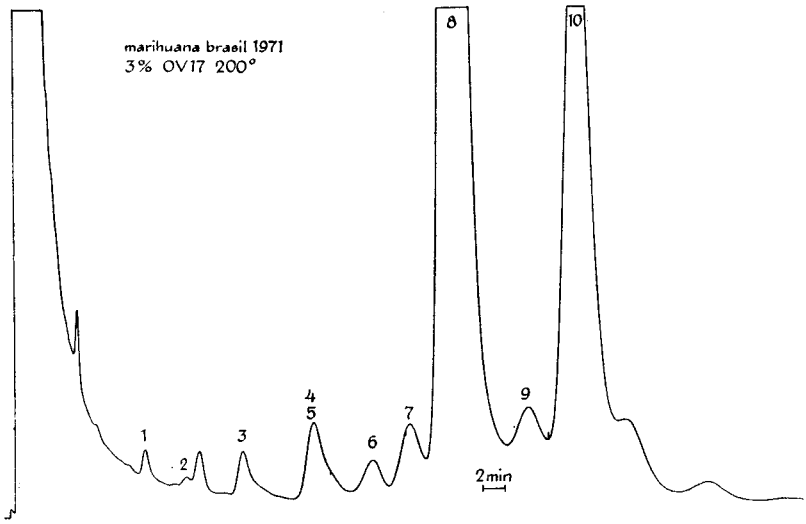

FIG. 1. Gas chromatogram of Brasil marihuana extract.

immediately gave rise to the question whether homologues with a methyl or a heptyl side-chain also exist in nature, assuming that in biogenesis the side-chain is formed by acetate fragments. We now wish to describe the occurrence of the homologues of Δ^1-THC, CBD and CBN having a methyl side-chain in hashish and marihuana. These have not been identified in cannabis preparations before. To indicate the differences as well as the similarities between the homologues, we have added to the abbreviations Δ^1-THC, CBD and CBN the terms -C1, -C3 and -C5, corresponding to the methyl-, the n-propyl- and the n-pentyl side-chain respectively.

MATERIALS AND METHODS

Materials

Lebanese hashish and Brasil marihuana were powdered and extracted with n-hexane for 10 min in a homogenizer. After filtration of the extracts, most solvent was evaporated to give suitable concentrations for gas chromatography.

Gas chromatography

A Hewlett-Packard 402 gas chromatograph with flame-ionization detector was used. Glass columns, 1·80 m, i.d. 4 mm, were packed with OV 17 3% on Gaschrom Q, 60–80 mesh. Oven 200°; injection block 250°; detector 250°. Nitrogen flow rate 20 ml/min; hydrogen flow 30 ml/min; air-flow 150 ml/min.

Gas chromatography-mass spectrometry

An LKB 9000 combined gas chromatograph-mass spectrometer was used. Glass columns, 1·50 m, i.d. 4 mm, were packed with OV 17 3% on Gaschrom Q, 60–80 mesh. Oven 200°; separator 220°; ion source 250°. Repetitive mass spectra were taken at 20, 18, 16, 14, 12 and 10 eV during the elution of a component from the gas-chromatograph, recorded by total ion current at 20 eV. The trap current was 60 μA, the accelerating voltage 3·5 kV. Gas chromatograms on this apparatus showed the same elution pattern as on the Hewlett-Packard 402. The mass spectra obtained were normalized and the relative intensity of a certain mass fragment was plotted versus the electron voltage. Typical graphs were obtained as shown in Figs 2, 3 and 5.

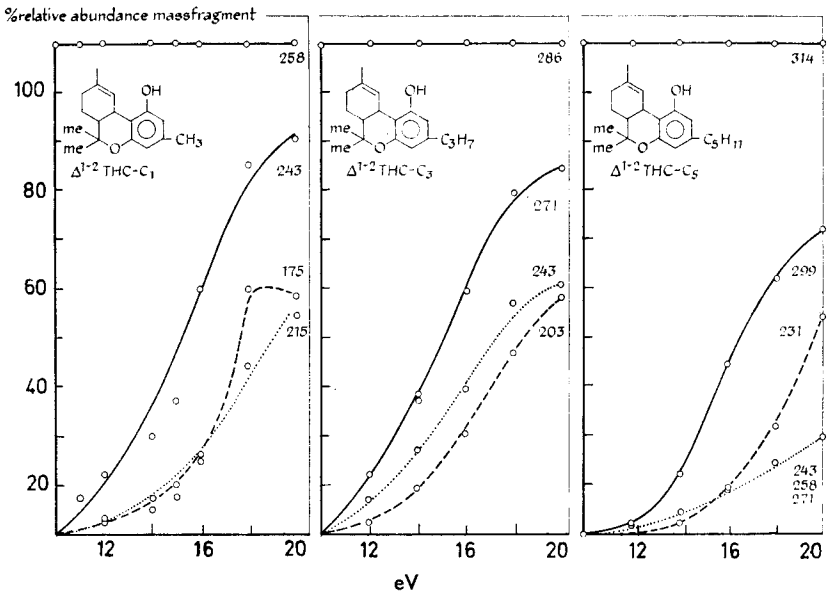

FIG. 2. Electron voltage-mass fragment intensity graphs of peak numbers 1, 3 and 10 in the gas-chromatogram of Brasil marihuana extract.

RESULTS

In Fig. 1 the gas chromatogram of the Brasilian marihuana extract is shown. From all peaks the electron voltage-mass fragment intensity graphs were plotted and some of these are represented in Figs 2 and 3. The two major peaks (8 and 10) were identified as representing Δ^1-THC-C5 and CBN-C5 respectively. In the two graphs (Fig. 2), corresponding to peak numbers 1 and 3 in the gas chromatogram, the shape of the curves very closely resemble those of Δ^1-THC-C5, the only difference being a constant mass fragment 28 or 56 less. As we know that the main fragmentation takes place in the alicyclic ring system of the cannabinoid molecules at the electronvoltages applied (Vree & others, 1971), we conclude from these graphs that this part of these molecules is identical for the three compounds. Previously (Vree & others, 1971) we concluded that the graph with fragments 28 less than Δ^1-THC-C5 must represent the THC-homologue having a propyl side-chain (Δ^1-THC-C3; M = 286). Referring to that, the only possible structure for the compound with mass fragments 56 less than Δ^1-THC-C5, corresponds to the THC-homologue with a methyl side-chain (Δ^1-THC-C1; M = 258). By analogy, peak number 2 in the gas chromatogram could be identified to represent the homologue of CBN having a methyl side-chain, the curves of mass fragments 254 and 239 having the same shape as those of CBN-C5 and CBN-C3 with differences of 56 and 28 respectively (CBN-C1; M = 254) (Fig. 3). On this column the cannabichromene and the CBN-C3 coincide, but the latter could easily be identified because of the characteristic mass fragments 282 and 267.

Thus, in the Brasil marihuana sample the main constituents Δ^1-THC-C5 and CBN-C5 are accompanied by their homologues with an n-propyl or a methyl side-chain. As no CBD-C5 could be detected it does not seem reasonable to expect any CBD-C3 or CBD-C1 in this sample. However, in a hashish sample of Lebanese origin, in which the CBD-C5 is one of the main constituents, we were able to identify the CBD-C3 and also

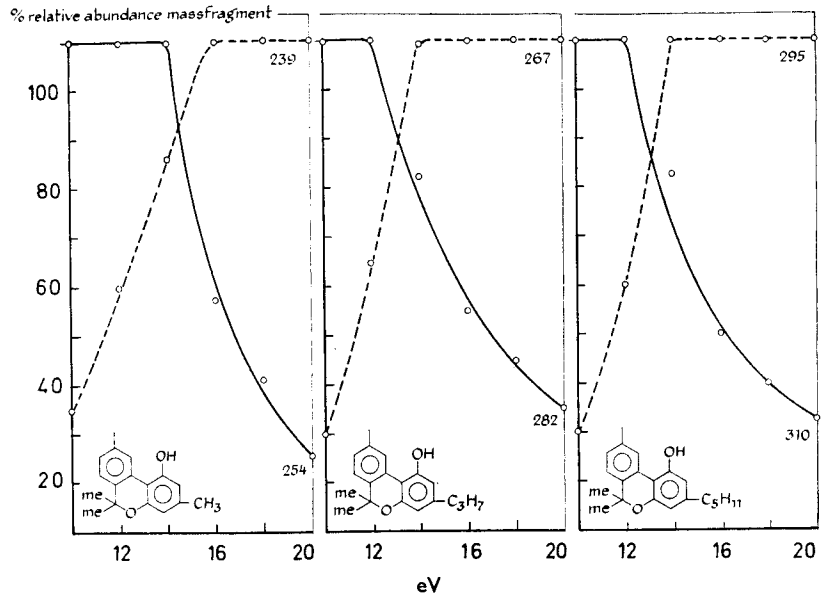

FIG. 3. Electron voltage-mass fragment intensity graphs of peak numbers 2, 4 and 10 in the gas-chromatogram of Brasil marihuana extract.

the homologue with a methyl side-chain (CBD-C1; M = 258). The gas chromatogram of this sample is shown in Fig. 4. The electronvoltage-mass fragment intensity graphs of peak-numbers 1, 3 and 6 show a striking resemblance (Fig. 5), but the differences are again fragments 28 and 56. Most characteristic is the shape of fragments 246, 218 and 190, which are due to CBD-C5, CBD-C3 and CBD-C1 respectively. In this Lebanese sample Δ^1-THC-C5 and CBN-C5 are also present in relatively large amounts and the occurrence of their propyl and methyl side-chain homologues was established in the same way as in the Brasil marihuana.

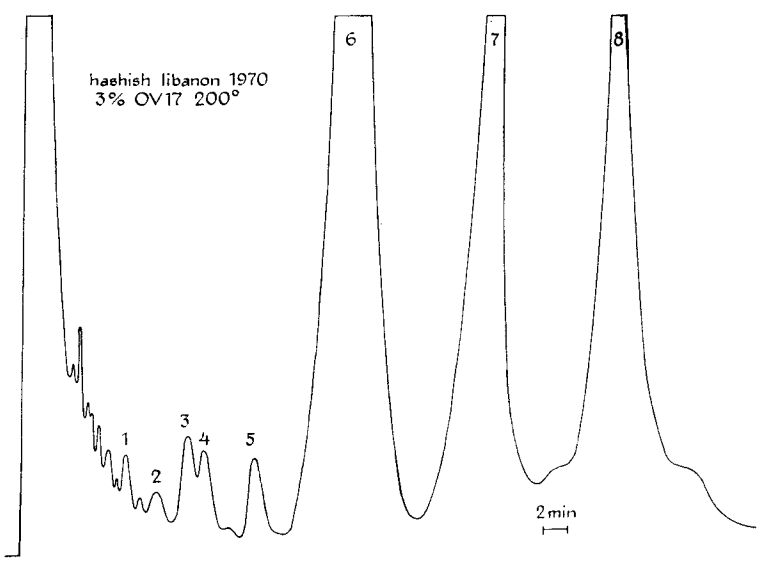

FIG. 4. Gas chromatogram of Lebanese hashish extract.

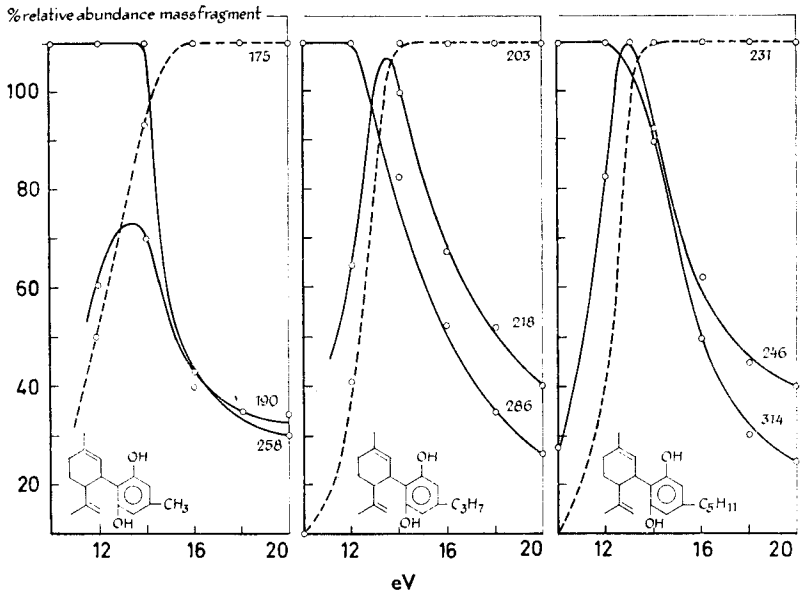

FIG. 5. Electron voltage-mass fragment intensity graphs of peak numbers 1, 3 and 6 in the gas-chromatogram of Lebanese hashish extract.

DISCUSSION

It seems reasonable on basis of the above observations and the analysis of several other hashish and marihuana samples, to state that the major cannabis constituents, bearing an n-pentyl side-chain, are generally accompanied by their homologues with an n-propyl and with a methyl side-chain. We must consider them to be natural constituents of hashish and marihuana. From the biogenetic point of view their existence is not very surprising, as alkylresorcinols, differing by two carbon atoms in the side-chain (formed by acetate fragment synthesis) are widespread in plants (Bullock, 1965; Soos, 1967). These resorcinol derivatives condense with suitable terpenoid moieties (e.g. geranyl pyrophosphate) to give the cannabinoids identified. So the compounds with an n-pentyl side-chain are derived from olivetol (5-n-pentylresorcinol), those with an n-propyl side-chain from divarinol (5-n-propylresorcinol) and those with a methyl side-chain form orcinol (5-methylresorcinol). The propylcannabinoids have been discovered recently. Vollner & others (1969) isolated the CBD-C3, Merkus (1971) found CBN-C3, Gill (1971) isolated Δ^1-THC-C3 and he found it biologically active in several aspects, but always less so compared with the Δ^1-THC-C5 (Gill & Paton 1970; Gill, Paton & Pertwee, 1970).

Until now the methyl cannabinoids have not been recognized as natural constituents of cannabis. The Δ^1-THC-C1 (Petrzilka, Haefliger & Sikemeier (1969) and the CBD C1 (Korte, Dlugosch & Claussen, 1966) have been synthesized, but data on biological activity of these compounds are not available. From a study of the activity of a series of synthetic Δ^3-THC homologues (Adams, Loewe & others, 1941), the Δ^3-THC-C1 appeared to be much less active than the Δ^3-THC-C5 and the Δ^3-THC-C3. By analogy, and taking into account the fact that the Δ^1-THC-C3 is less active than Δ^1-THC-C5, we expect the Δ^1-THC-C1 to possess a lower biological activity than the other Δ^1-THC homologues. In most hashish and marihuana samples the propyl and

methyl cannabinoids are present only in small concentrations. However, in some samples of Asian origin we found relatively large quantities of Δ^1-THC-C3, and because of its activity it would be expected that this should contribute substantially to the biological activity of these samples. No sample we have examined so far contained large concentrations of the methyl cannabinoids. For this reason, and because of the assumed low activity, we do not expect a substantial contribution of Δ^1-THC-C1 to the overall biological activity of hashish and marihuana.

Finally, we would like to propose trivial names for the new class of cannabinoids. They are derived from orcinol and we therefore propose: Δ^1-tetrahydrocannabiorcol (Δ^1-THC-C1), cannabidiorcol (CBD-C1) and cannabiorcol (CBN-C1). See Table 1.

Table 1. *Nomenclature of cannabinoid homologues.*

Δ1-THC-Cx	Olivetol (C5) Δ^1-tetrahydrocannabinol	Divarinol (C3) Δ^1-tetrahydrocannabivarol (Δ^1-tetrahydrocannabivarin)	Orcinol (C1) Δ^1-tetrahydrocannabiorcol
CBD-Cx	cannabidiol	cannabidivarol (cannabidivarin)	cannabidiorcol
CBN-Cx	cannabinol	cannabivarol (cannabivarin)	cannabiorcol

This nomenclature is in agreement with Δ^1-tetrahydrocannabivarol for Δ^1-THC-C3 proposed by Gill (1971). The propyl cannabinoids are derived from divarinol and so the names for the other compounds should be cannabidivarol (CBD-C3) and cannabivarol (CBN-C3). However, the latter two constituents were first called cannabidivarin (Vollner & others, 1969) and cannabivarin (Merkus, 1971). Although we also used this nomenclature in our previous paper (Vree & others, 1971), we prefer trivial names ending in *-ol* rather than those ending in *-in*; the latter would suggest that we are dealing with some kind of an amine rather than an alcohol or a phenol.

Acknowledgments

The authors wish to thank Dr. E. A. Carlini, Faculdade de Ciências Médicas da Santa Casa, Sao Paulo, Brasil, for the Brasil marihuana and to Dr. A. H. Witte, Forensic Laboratory, Ministry of Justice, The Hague, The Netherlands, for various hashish and marihuana samples.

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