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Identification of cannabicyclol with a pentyl or propyl side-chain by means of combined gas chromatography–mass spectrometry

Cannabicyclol occurs only in minor concentrations in natural marihuana and hashish samples. The compound was first isolated by KORTE AND SIEPER[1]. Later, GAONI AND MECHOULAM[2] and CLAUSSEN *et al.*[3] isolated the compound and elucidated its molecular structure. The structure was confirmed by the synthesis of the compound by CROMBIE *et al.*[4]. GAONI AND MECHOULAM were also the first to determine the retention time in gas chromatography (GC) on a column of 2 % OV-17. In our experiments, we found that cannabicyclol was the first compound to be eluted from the OV-17 column with a molecular weight of 314. The compound could be separated from the other well known hashish constituents by GC, and by combining GC and mass spectrometry (MS) it was simple to obtain the mass spectrum of cannabicyclol. Although the fragmentation pattern of the cyclol is not well understood, the mass spectrum can be distinguished clearly from the mass spectra of other cannabinoids, for example, cannabichromene, cannabidiol, 1,6- and 1,2-tetrahydrocannabinol, all of which have a molecular weight of 314 and fragments *m/e* 299, 271, 258, 246, 243 and 231 in common.

It is known that the main constituents with a pentyl side-chain are accompanied by their homologues bearing a propyl and a methyl side-chain[5–10]. It might therefore be expected that cannabicyclols, with propyl and methyl side-chains, also exist. The detection of these compounds depends largely upon the availability of a hashish or marihuana sample in which the C_3 and C_1 compounds are present in such a concentration that they can be detected and identified.

Materials and methods

An ether extract of a sample of Congo marihuana was injected into the GLC HP 402 and into the GC–MS LKB 9000 instruments. In each instance, the columns were filled with 3 % OV-17 on Gas-Chrom Q, 60–80 mesh. The carrier gas was helium at a flow-rate of 20 ml/min; temperature of the oven 180°, of the separator 240°, and of the ion source 290°; accelerating potential 3.5 kV; trap current 60 μA; and the electron energies 20, 18, 16, 14, 12 and 10 eV during the elution of a peak. The spectra obtained were normalized and the relative abundances of a particular mass fragment were plotted against the electron energy used[8–10]. The propyl side-chain is indicated with C_3, the methyl side-chain with C_1 and the pentyl side-chain with C_5. Retention times are measured in centimetres; the fictive retention is calculated with 1-2-THC-C_5* as the reference compound with a fictive retention of 100 (ref. 11).

Results

Mass spectrometry. The mass spectrum of cannabicyclol-C_5 obtained from the marihuana samples was identical with that of the compound isolated by GAONI AND MECHOULAM[2]. The mass spectra at 20 eV of cannabicyclol with a pentyl (C_5) and propyl (C_3) side-chain are given in Table I.

* Δ 1,2-THC = 1,2-tetrahydrocannabinol; monoterpenoid numbering (in this paper Δ is omitted).

TABLE I

MASS SPECTRA OF CANNABICYCLOLS AT 20 eV

Mass fragment m/e	*Relative abundance (%)*	
	Cannabicyclol-C_5	*Cannabicyclol-C_3*
314	33	
299	7.5	
286		20
271	8	6
258	7	
246	3	
243	5	
231	100	
230		2
218		2
203		100
174	5	

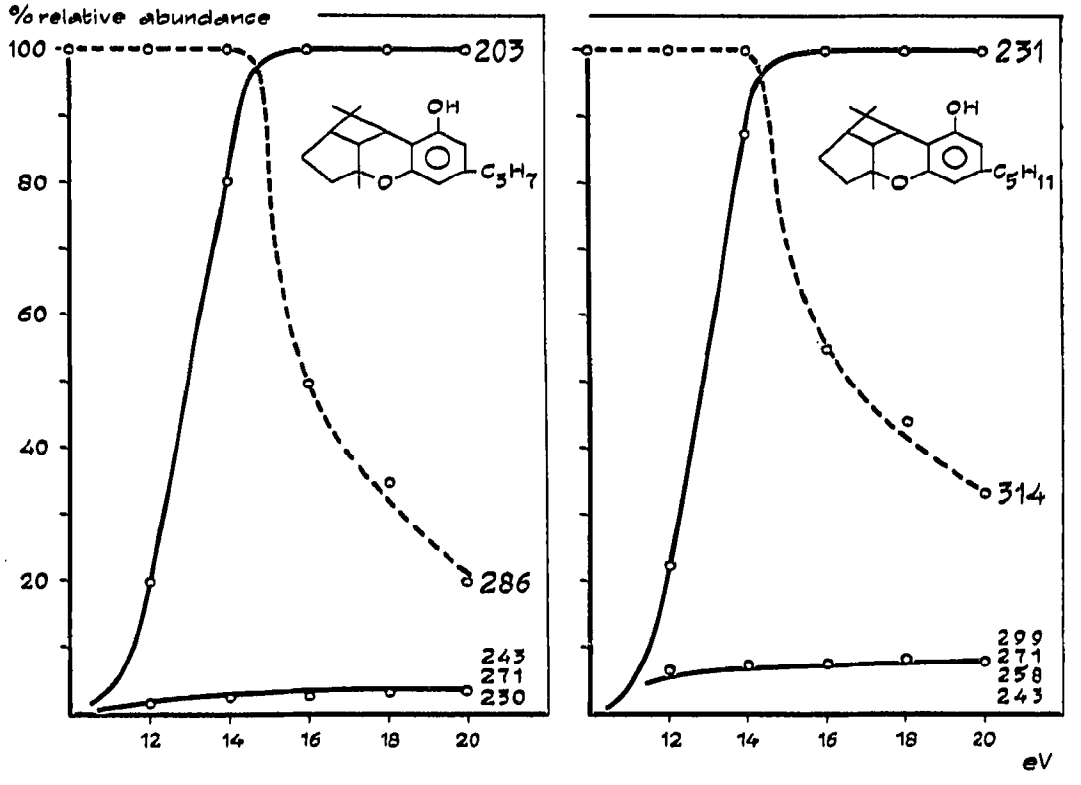

Fig. 1. Relative abundance of the mass fragments plotted against the electron energy used.

When the relative abundances of the mass fragments are plotted against the electron energy used, as shown in Fig. 1, the graphs have the same shape but the corresponding mass fragments have a constant difference of 28, which, by analogy

with THC-C_3, CBD-C_3* and CBN-C_3** and other compounds that have structural differences only in the side-chain (II), can be explained only by differences in the side-chain[8-10,12]. Also, the "crossing points" at 14 eV of the line of *m/e* 314 with that of *m/e* 231, and of *m/e* 286 with *m/e* 203, provides further evidence that apart from the side-chains, the molecular structures are identical[10]. For instance, the crossing point of the lines of the same fragments *m/e* of cannabichromene-C_5 and -C_3 is at 10 eV.

Cannabicyclol-C_5 can also be distinguished from cannabichromene-C_5, *e.g.*, by the presence of the fragments *m/e* 299, 271, 258 and 243 in equal relative abundances (6–8 %). The latter fragments are not present in cannabichromene-C_5. The same observation holds for the presence of the mass fragments *m/e* 271, 243, 230 and 218 in cannabicyclol-C_3, their being absent in cannabichromene-C_3.

Gas chromatography. Gas chromatography provides some other evidence for the existence of cannabicyclol-C_3. As was observed earlier, the ratio of the retention times of the cannabinoid homologues, beating a methyl, a propyl and a pentyl side-chain, is constant and almost independent of the cannabinoid and instrumental settings[10,11,13].

TABLE II

RELATIVE RETENTION TIMES

Compound	*Retention*[a] *(cm)*	*Fictive retention*[b]	*Relative retention time*
Cannabicyclol-C_3	4.6	14.3	1.00
Cannabicyclol-C_5	9.8	30.0	2.09
1,2-THC-C_3	—	50.0	1.00
1,2-THC-C_5	32.5	100.0	2.00
CBN-C_3	—	60.0	1.00
CBN-C_5	—	126.0	2.10

[a] Gas chromatogram of Congo marihuana on 3 % OV-17 at 180°.
[b] See ref. 11.

From Table II, it can be derived that the ratio of the retention time of cannabicyclol-C_3 to that of cannabicyclol-C_5 fits well with the results obtained for the known structures THC-C_3/C_5, CBN-C_3/C_5, etc.

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* CBD = cannabidiol.
** CBN = cannabinol.

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