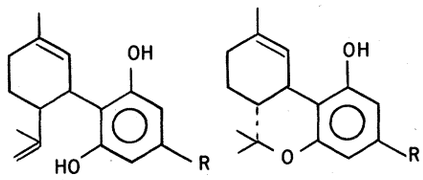
Cannabinoids with a Propyl Side Chain in Cannabis: Occurrence and Chromatographic Behavior

Abstract. *Neutral cannabinoids with a pentyl side chain—for example, cannabidiol, tetrahydrocannabinol, and cannabinol—are generally accompanied by homologs with a propyl side chain, of which at least one has psychotropic activity. Samples of hashish and marihuana from Asia especially sometimes have abundant amounts of propyl cannabinoids, the quantities being of the same order as that of the accompanying pentyl cannabinoids. Detection and identification of the propyl and pentyl cannabinoids in gas chromatography and thin-layer chromatography is discussed.*

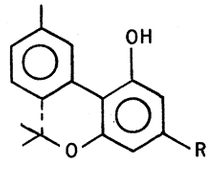
The chemical composition of hashish and marihuana has been well studied (*1*). Cannabidiol (**1**), Δ^1-tetrahydrocannabinol (**2**) (monoterpenoid numbering), and cannabinol (**3**) were identified as the major neutral cannabinoids (*1*) of which Δ^1-tetrahydrocannabinol was found to be psychotropically active (*2*). The neutral cannabinoids can be accompanied by their respective acids, having a carboxyl group in one of the free positions at the aromatic ring. The cannabinoid acids are psychotropically inactive (*1*) and not very stable. Under the influence of light and heat, for example on smoking, decarboxylation to neutral cannabinoids rapidly occurs.

These cannabinoids can be regarded as monoterpenoids coupled with olivetol (5-*n*-pentylresorcinol), with the structural differences occurring in the terpenoid part of the molecule.



1, R = C_5H_{11} **2**, R = C_5H_{11}

1a, R = C_3H_7 **2a**, R = C_3H_7



3, R = C_5H_{11}

3a, R = C_3H_7

A new class of cannabinoids, which can be regarded as derived from divarinol (5-*n*-propyl-resorcinol) rather than of olivetol has been discovered. Vollner *et al.* (*3*) isolated the propyl analog of cannabidiol and proposed the name of cannabidivarin (**1a**); Gill *et al.* (*4*) isolated Δ^1-tetrahydrocannabivarin (**2a**), and Merkus (*5*) found cannabivarin (**3a**). Although these trivial names are in common use, the following abbreviations are more satisfactory for our work. Cannabidiol, pentyl-CBD; Δ^1-tetrahydrocannabinol, pentyl-Δ^1-THC; cannabinol, pentyl-CBN; cannabidivarin (the propyl homolog of CBD), propyl-CBD; Δ^1-tetrahydrocannabivarin, propyl-Δ^1-THC; and cannabivarin, propyl-CBN. These notations indicate the differences as well as the similarities between the various cannabinoids, and the system can also be used successfully if other propyl homologs are discovered. The above abbreviations will be used here.

Propyl-Δ^1-THC was found to be 4.8 times less active than pentyl-Δ^1-THC in producing cataleptic states in mice (*4*). No information exists on the activity of the other propyl cannabinoids, but the corresponding pentyl components are psychotropically inactive on smoking and on intravenous injection (*2*, *6*). However, on intracerebral injection, both pentyl-CBD and pentyl-CBN showed about the same activity in mice as did equal doses of pentyl-Δ^1-THC (*6*).

In view of these developments procedures are required for the detection, identification, and evaluation of the propyl cannabinoids in *Cannabis* products. We have identified propyl cannabinoids by a new method of combined gas chromatography and mass spectrometry (*7*). We now report the chromatographic behavior of the components in gas chromatography and thin-layer chromatography and describe their occurrence in nature.

Samples of hashish and marihuana from different sources were used and were obtained from police seizures. Resin (0.1 g) or herb (0.5 g) were dried, powdered, and extracted twice with fresh 5-ml portions of chloroform. The combined filtered extracts were concentrated to a volume of about 2 ml by evaporation under reduced pressure. Various samples of Extractum Cannabis (*8*), which is an ethanol percolate of *Cannabis sativa* var. *indica*, were also used. A portion (0.1 g) of this extract was dissolved in 1 ml of acetone and then filtered. The identity of the cannabinoids was confirmed by combined gas chromatography and mass spectrometry (*7*).

A typical gas chromatogram of a hashish sample is shown in Fig. 1 (*9*). The major peaks could be identified as propyl-CBD, propyl-Δ^1-THC, propyl-CBN, pentyl-CBD, pentyl-Δ^1-THC, and pentyl-CBN, respectively. The propyl cannabinoids are eluted first, and their separation sequence is similar to that of the pentyl homologs. Under the conditions used, cannabinoid acids, if present, would decarboxylate and thus contribute to the peak of their respective neutral cannabinoids. Decarboxylation can be prevented by making trimethylsilyl derivates, but after silylation of the sample no silylated acids appeared in the chromatogram. The peaks in Fig. 1 can therefore be attributed to the presence of neutral cannabinoids only.

As compared to gas chromatography, thin-layer chromatography, another important technique in *Cannabis* analyses, is less useful for separating the six cannabinoids. We tested a neutral, an alkaline, and a reversed phase system and found various overlappings occurring in all three (Fig. 2) (*10*). The propyl cannabinoids elute more slowly than their corresponding pentyl homologs; this often results in coinciding of one of the slower components of the pentyl class with one of the faster-moving spots from the propyl class. Thus, in the neutral and alkaline systems the six components show up as four spots, of which the highest represents a single pentyl component, the lowest a single propyl com-

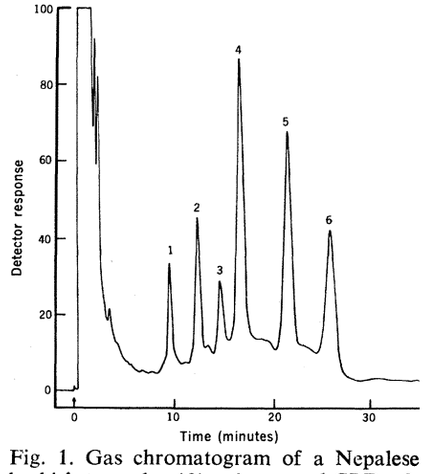


Fig. 1. Gas chromatogram of a Nepalese hashish sample (*9*): 1, propyl-CBD; 2, propyl-Δ^1-THC; 3, propyl-CBN; 4, pentyl-CBD; 5, pentyl-Δ^1-THC; and 6, pentyl-CBN.

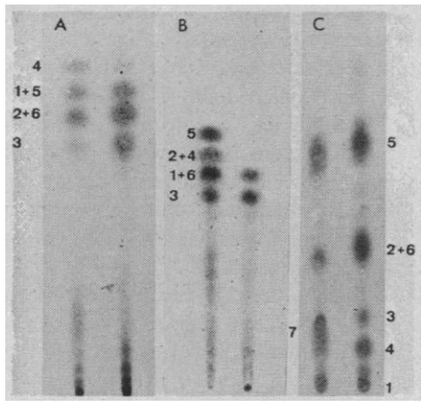


Fig. 2. Thin-layer chromatograms of hashish samples (*10*). (A) Solvent system petroleum ether, ether (80 : 20). (B) Solvent system hexane, dioxane (75 : 25) in combination with a trough with ammonia. (C) Solvent system cyclohexane on dimethylformamide-impregnated plates: 1, propyl-CBD; 2, propyl-Δ^1-THC; 3, propyl-CBN; 4, pentyl-CBD; 5, pentyl-Δ^1-THC; 6, pentyl-CBN; and 7, pentyl-Δ^1-THC acid.

ponent, and the other two a mixture of one pentyl and one propyl component each. In the reserved phase system propyl-Δ^1-THC coincides with pentyl-CBN, while propyl-CBN lies just ahead of pentyl-CBD or may be overlapped by high concentrations of the latter.

The presence of Δ^1-THC acid *A*, if any, will make the detection of propyl-CBN or pentyl-CBD even more difficult as the elongated spot of the acid will overlap the other two. In the reserved phase system the migration distances of the spots are easily affected by the degree of impregnation. With shorter drying times or at high relative humidities the position of all spots moves upward, but the separation sequence does not change. The colors of the cannabinoids after spraying with fast blue salt B are the same for pentyl and propyl components: scarlet red for the THC's, orange for the CBD's but deepening to red at high concentrations, and purplish red for the CBN's.

The occurrence of the propyl cannabinoids in nature seems to depend on the origin of the samples. In those from Asian countries like India, Nepal, and Pakistan we usually detected abundant amounts of propyl components (Fig. 1). In a few samples the propyl cannabinoids even showed larger peak areas than their accompanying corresponding pentyl analogs. Exact quantification of the propyl cannabinoids could not be performed because suitable reference materials were not available. However, because propyl-Δ^1-THC is psychotropically active, large quantities of this substance would contribute considerably to the total activity of a *Cannabis* sample. In general, we found peak areas of propyl-Δ^1-THC and propyl-CBD larger than that of propyl-CBN, but we found no correlation between the amount of a certain propyl component and that of its accompanying pentyl homolog. For example, a high concentration of propyl-Δ^1-THC is not necessarily accompanied by a similar concentration of pentyl-Δ^1-THC, and vice versa. A small number of Asian samples indicated the presence of minor amounts of the propyl analog of cannabichromene and the propyl analog of cannabicyclol, but conclusive evidence is not yet available as we lack sufficiently pure reference samples of the corresponding pentyl components. The availability of such references is a prerequisite for the identification of propyl cannabinoids by means of combined gas chromatography and mass spectrometry (*7*).

Samples from Middle Eastern and Mediterranean countries also contained propyl cannabinoids, but in much lower concentration than Asian samples. In gas chromatograms taken under conditions to suitably record pentyl cannabinoids the propyl components were usually difficult to distinguish from the background. However, when higher quantities were injected, detection and identification could still be performed by combined gas chromatography and mass spectrometry.

So far, we have been able to detect propyl cannabinoids in all samples investigated, so that these components seem to be natural constituents in addition to the pentyl cannabinoids. Samples from the Americas were not available for testing (*11*).

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References and Notes

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7. T. B. Vree, D. D. Breimer, C. A. M. van Ginneken, J. M. van Rossum, R. A. de Zeeuw, A. H. Witte, *Clin. Chim. Acta* **34**, 365 (1971).
8. Prepared according to the *Netherlands Pharmacopoeia* (Algemene Landsdrukkerij, The Hague, ed. 5, 1926).
9. Becker 409 with flame ionization detector; stainless steel columns (2 mm by 2 m) packed with 5 percent SE-30 on DMCS treated, acid-washed Chromosorb G, 80 to 100 mesh; carrier gas nitrogen, 20 ml/min, inlet pressure 2.5 kg/cm²; injection block 275°C, oven 230°C, detector 275°C; sample size 1 to 2 μl. Trimethylsilyl derivates were obtained by evaporating 0.5-ml samples to dryness under a stream of nitrogen and dissolving the dried samples in 0.5 ml of Tri-Sil (Pierce). After shaking and standing for 5 minutes, the solution is ready for injection.
10. Plates had layers of silica gel G (0.25 mm) (Merck). Solvent systems were petroleum ether (b.p. 40° to 60°C), ether (80 : 20) as described [G. Machata, *Arch. Toxikol.* **25**, 19 (1969)]; hexane, dioxane (75 : 25) in combination with a trough with 10 ml of 25 percent ammonia at the botttom of the chamber; cyclohexane on dimethylformamide-impregnated plates [F. Korte and H. Sieper, *J. Chromatogr.* **13**, 90 (1964)], and the procedure described by F. W. H. M. Merkus [*Pharm. Weekblad* **106**, 49 (1971)]. All solvents were reagent grade and compositions are given by volume. Development took place in unsaturated chambers to a height of 15 cm over the starting points. Visualization was effected with a 0.5 percent solution of *o*-dianisidine tetrazolium chloride (fast blue salt B, Merck) in water. Temperatures ranged from 20° to 22°C and the relative humidity ranged from 22 to 55 percent. In these ranges reproducible R_F values were obtained with the first two solvent systems; in the reserved phase system R_F values may vary more or less, depending on the drying conditions after the impregnation, but the separation sequence does not change. Photographs of authentic chromatograms were made after removal of the layer from the glass plate by means of Neatan (Merck).
11. After completion of our manuscript a report by Merkus (*12*) has appeared, dealing with some chromatographic properties of propyl cannabinoids. Mass spectrometry, most probably with a 70-ev beam was used to identify the cannabinoids. However, as was pointed out earlier (*7*), the reliability of this procedure in *Cannabis* research is questionable. This holds in particular for the identification of the component designated tetrahydrocannabivarin, which was isolated by thin-layer chromatography (*12*). In our experiments on dimethylformamide-impregnated plates we found propyl-THC (tetrahydrocannabivarin) always completely coinciding with pentyl-CBN. In addition, we frequently encountered a different component in the same area just ahead of the spot containing propyl-THC and pentyl-CBN. This unknown component could be identified as the propyl analog of cannabichromene (unpublished). The mass spectra at 70 ev of the propyl analog of cannabichromene and propyl-THC are quite similar, so that differentiation between these two can hardly be obtained by this technique. Gas chromatography combined with mass spectrometry at varying electron beam energies (*7*) should be recommended in such cases.
12. F. W. H. M. Merkus, *Nature* **232**, 579 (1971).
13. We thank Dr. A. H. Witte, Laboratory of Forensic Sciences, Ministry of Justice, The Hague, for various hashish and marihuana samples and Dr. A. Segelman, University of Pittsburgh, for an authentic sample of pentyl-Δ^1-THC.

9 September 1971; revised 19 October 1971