Journal of Chromatography, 120 (1976) 343–348

CHROM. 8960

CHROMATOGRAPHIC SEPARATION OF CANNABINOIDS AND THEIR MONOOXYGENATED DERIVATIVES

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(Received December 1st, 1975)

SUMMARY

Δ^1-Tetrahydrocannabinol, Δ^6-tetrahydrocannabinol, cannabinol, cannabidiol and several of their monooxygenated derivatives have been separated from each other by a combination of liquid, thin-layer and gas chromatography. Retention volumes (on Sephadex LH-20), R_F values and retention times can be recorded, and may provide guidance in the separation and identification of these known cannabinoids.

INTRODUCTION

Δ^1-Tetrahydrocannabinol (Δ^1-THC) is regarded[1] as the major naturally occurring psychoactive component of *Cannabis sativa* L. The isomeric Δ^6-tetrahydrocannabinol (Δ^6-THC), usually present in small amounts, has an activity[1] similar to that of Δ^1-THC. Two other major compounds present in cannabis are cannabinol (CBN) and cannabidiol (CBD). These two compounds have been reported to lack psychotomimetic activity[1]. However, recent evidence showed that CBN when administered to man shows a slight psychoactive effect[2], and that CBD caused interference with the action of Δ^1-THC in man[3]. The metabolic patterns of these cannabinoids are complex[4]. In order to be able to identify metabolites having similar properties and which are produced in microgram quantities, synthetic reference compounds are required together with a variety of separation techniques.

In this paper we report the separation of cannabinoids and their monooxygenated metabolites by a combination of liquid chromatography on Sephadex LH-20, thin-layer chromatography (TLC) and gas chromatography (GC). Only one dioxygenated metabolite (6α,7-dihydroxy-Δ^1-THC) is discussed here; other dioxygenated metabolites of CBN[5] and CBD[6] will be reported elsewhere.

EXPERIMENTAL

The compounds used in this investigation were either synthetic references[4,7,8] or metabolites[7,9,10] obtained from previous work.

Liquid chromatography

For liquid chromatography, a column of Sephadex LH-20 (70 × 1 cm) was used with light petroleum (b.p. 40–60°)–chloroform–ethanol (10:10:1) as the eluent[11]. The flow-rate was adjusted to 0.15 ml/min and the mixtures of cannabinoids (*ca.* 50 μg of each) were chromatographed under the same conditions. In each run, 3″-hydroxy-Δ^6-THC (elution volume, 90–100 ml) was used as an internal standard. Fractions of 2 ml were collected. The fractions were evaporated to 0.5 ml. Aliquot portions (25 μl) were spotted on to a filter paper and sprayed with Fast Blue B salt in order to localize the cannabinoids. The compounds were then identified by TLC and GC as described below.

Thin-layer chromatography

Unless stated otherwise, TLC was carried out on pre-coated silica gel F plates (E. Merck, Darmstadt, G.F.R.; 0.25-mm thickness, 5 × 10 cm) which were developed in diethyl ether–light petroleum (3:2) and visualized with 0.2% Fast Blue B salt in 2 *N* aqueous sodium hydroxide.

Gas chromatography

A Varian Aerograph Model 2100 chromatograph equipped with a hydrogen flame-ionization detector (FID) and a glass column (6 ft. × 0.125 in.) packed with 2% SE-30 ultraphase on Gas-Chrom Q (125–150 mesh) was employed. The injector and detector temperatures were maintained at 270°, while the column was operated isothermally at 250°. The carrier gas (nitrogen) flow-rate was 25 ml/min. The hydrogen flow-rate was the same as that of nitrogen, while the oxygen flow-rate was 200 ml/min. Some compounds were derivatized as their trimethylsilyl (TMS) ethers according to the method described previously[8].

RESULTS AND DISCUSSION

Liquid chromatography

The separation of the cannabinoids and their metabolites on Sephadex LH-20 is shown in Fig. 1. In the CBD series, where all of the five side-chain hydroxylated metabolites were available, the order of separation was 2″-, 1″-, 3″-, 4″- and 5″-hydroxylated compound. Although some of the side-chain hydroxylated metabolites were lacking in the other series, the order of separation observed within each series was the same as that given above.

Compounds hydroxylated in the 3″- and 4″-position in all of the series were poorly separated, as were metabolites like 7-hydroxy-, 6β-hydroxy- and 6α-hydroxy-Δ^1-THC. However, these metabolites could be separated by TLC (Fig. 2) and/or by GC (Table I) under the conditions described in the Experimental section. This overlapping of compounds might be overcome by high-pressure liquid chromatography. However, the described Sephadex column has wide application[11].

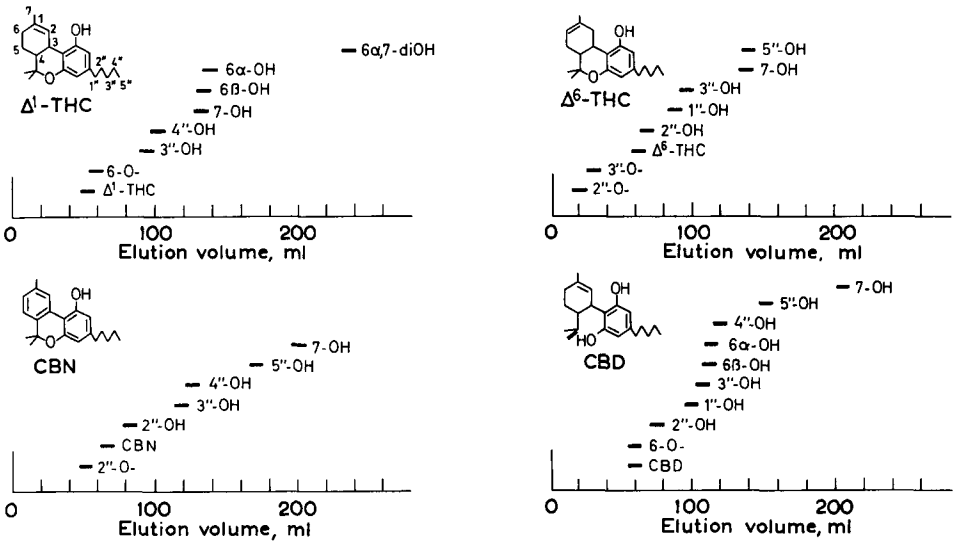

Fig. 1. Elution patterns of Δ^1-THC, Δ^6-THC, CBN, CBD and their derivatives on Sephadex LH-20. Abbreviations as exemplified by the Δ^1-THC series: 6-O- is 6-oxo-Δ^1-THC; 3″-OH is 3″-hydroxy-Δ^1-THC; 6α,7-diOH is 6α,7-dihydroxy-Δ^1-THC, etc. For the conditions used, see Experimental section.

Thin-layer chromatography

The TLC separation of the cannabinoids is depicted in Fig. 2. Pre-coated silica gel F plates developed in diethyl ether–light petroleum (3:2) were found to be a satisfactory system. Compounds hydroxylated in the 3″- or 4″-position also showed similar properties on TLC, but could be well separated by repeated chromatography (2 or 3 times) using the same solvent system. The 7-hydroxy compound in each series showed similar R_F values to the side-chain hydroxylated metabolites (3″- and 4″-hydroxy). However, on aluminium oxide plates (E. Merck) developed in 1% Methanol in chloroform, the 7-hydroxy compound had a much lower R_F value than the side-chain hydroxylated metabolites. In the Δ^6-THC and CBD series (Fig. 2), a partial resolution of the isomers of 1″-hydroxy-Δ^6-THC and 1″-hydroxy-CBD was observed but the compounds were not totally separated. The isomers could be well separated by repeated chromatography (3 times) using diethyl ether–light petroleum (2:5) as solvent system.

The limitation of the diethyl ether–light petroleum (3:2) solvent system arose with the dihydroxy metabolites. As seen from Fig. 2, 6α,7-dihydroxy-Δ^1-THC remained at the starting point. Acetone–chloroform (7:13) was found to be a suitable solvent system in this case and an R_F value of 0.44 was observed. 0.5% Methanol in diethyl ether (developed 2 or 3 times) has been used to separate dihydroxylated metabolites of CBN[5] and CBD[6] on silica gel F plates.

Gas chromatography

The GC patterns of the cannabinoid series were very similar. The retention times are given in Table I. In the CBD series, the side-chain hydroxylated metabolites had retention times in the sequence of 2″-, 1″-, 3″-, 4″- and 5″-hydroxy compound.

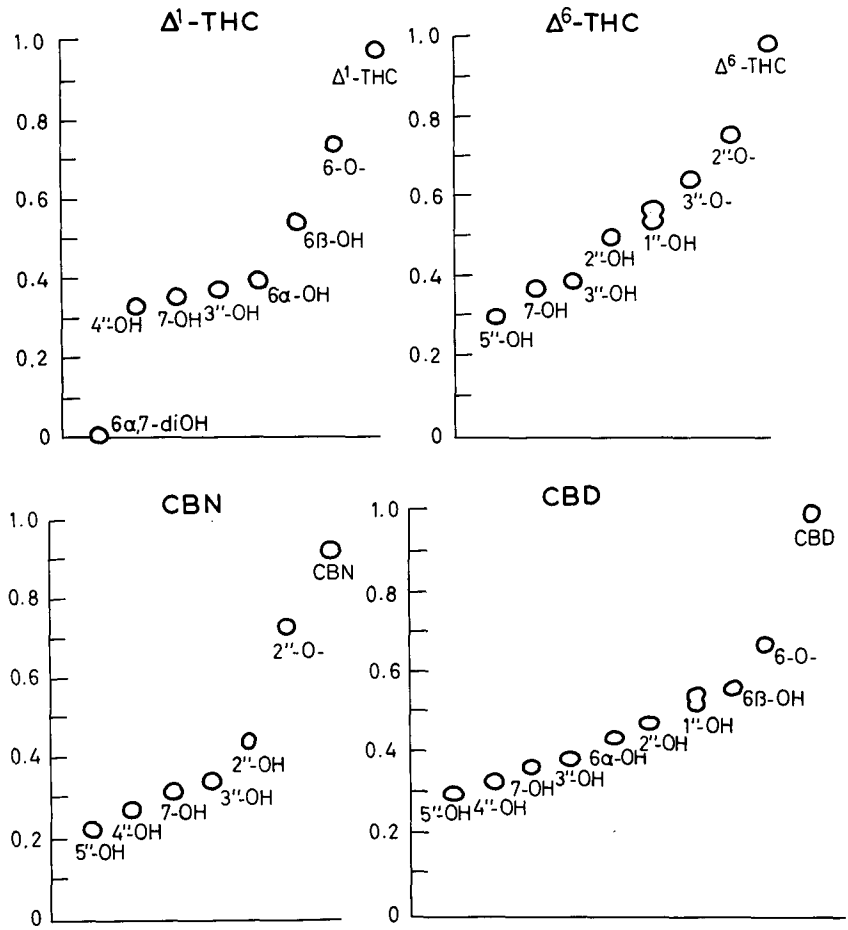

Fig. 2. Thin-layer separation of Δ^1-THC, Δ^6-THC, CBN, CBD and their derivatives. For the conditions used, see Experimental section.

This same order was indicated for side-chain hydroxylated compounds in the other series, although some compounds were lacking.

Compounds with the same or similar retention times could be separated as their TMS derivatives. In GC, the 3″- and 4″-hydroxy compounds showed similar properties, but could be separated as their TMS derivatives. In the Δ^6-THC series, the 6-oxo compound decomposed in GC unless it was silylated. Also, it was possible to obtain a chromatogram of 6α,7-dihydroxy-Δ^1-THC (Table I) only after silylation. We have previously shown[8] the usefulness of TMS derivatives for the identification of different side-chain hydroxylated cannabinoids.

The chromatographic behaviour on Sephadex LH-20, TLC and GC of the cannabinoids seem to be very similar from one series to another. Column (Sephadex LH-20) and GC separation patterns of the side-chain hydroxylated metabolites were in the same order. In both techniques the 2″-hydroxy derivative showed a lower retention volume or retention time than the 1″-hydroxy derivative, which was followed

TABLE I

GAS CHROMATOGRAPHIC SEPARATION OF SOME CANNABINOIDS ON A COLUMN OF 2% SE-30 AT 250°

Compound	*Retention time (min)*			
	Δ^1-THC	*Δ^6-THC*	*CBN*	*CBD*
underivatized	4.8	4.4	6.0	3.5
2″-O-	—	8.7	11.8	—
2″-OH	—	8.9	12.2	7.1
1″-OH	—	9.3	—	7.4
3″-O-	—	10.0	—	—
6β-OH	10.1	—	—	7.9
6α-OH	10 4	—	—	8.0
3″-OH	11.0	10.1	14.1	8.2
4″-OH	11.1	—	14.3	8.3
6-O-	—	—	—	8.6
7-OH	14.2	14.1	18.2	10.7
5″-OH	—	14.3	19.3	11.0
TMS ethers				
3″-OH	3.7	—	5.3	2.1
4″-OH	4.1	—	5.7	2.4
6-O-	4.4	—	—	2.8
7-OH	—	4.7	—	—
5″-OH	—	5.1	—	—
6α,7-diOH	4.5	—	—	—

by the 3″-, 4″- and 5″-hydroxy compounds. Thus by different combinations of liquid chromatography on Sephadex LH-20, of TLC and of GC as presented here, suitable separation systems for the cannabinoids and their monooxygenated derivatives can be devised.

ACKNOWLEDGEMENTS

We thank Professor R. Mechoulam, Dr. K. Leander and the National Institute of Drug Abuse for generous gifts of reference compounds. The interest of Professor F. Sandberg towards this work is highly appreciated. K.F. wishes to express her gratitude to the International Seminar in Chemistry, University of Uppsala, for the award of a research fellowship. This work was supported by the Swedish Medical Research Council.

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