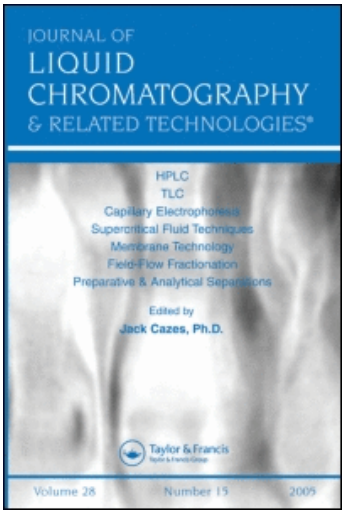

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SEPARATION OF TETRAHYDROCANNABINOL ISOMERS BY REVERSE-PHASE HIGH PRESSURE LIQUID CHROMATOGRAPHY

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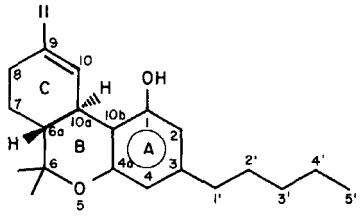
ABSTRACT

The separation of three closely related tetrahydrocannabinol isomers differing only in the position of the double bond in ring C was achieved by HPLC using a μBondapak C18 column and a ternary mobile phase of acetonitrile/tetrahydrofuran/water. Near base line resolution was obtained on the first pass through the column and complete resolution was accomplished after one recycle.

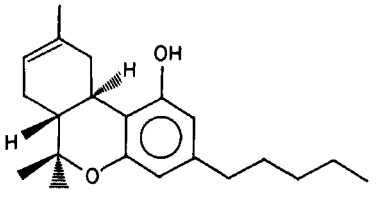
INTRODUCTION

Cannabis has been used as a therapeutic agent since ancient times in a wide variety of conditions including pain, glaucoma, nausea and respiratory ailments (1). However, since the plant contains over four hundered constituents, much of the pharmacological testing today is being carried out on individual naturally occurring cannabinoids or structurally related synthetic analogs.

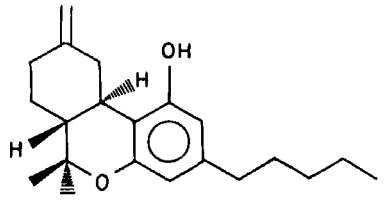
Δ^9-tetrahydrocannabinol (Δ^9-THC), Δ^8 -tetrahydrocannabinol (Δ^8-THC) and $\Delta^{9,11}$-tetrahydrocannabinol ($\Delta^{9,11}$-THC) are three tetrahydrocannabinol isomers that have received a great deal of attention and differ only in the position of the double bond in ring C. Of these, Δ^9-THC is the most active constituent of marijuana and remains the favorite cannabinoid for pharmacological and clinical studies. Δ^8 -THC is physiologically active but less potent than Δ^9 -THC (2) and has been found only in a few varieties of the plant. $\Delta^{9,11}$-THC is a synthetic analog differing from the other two by having a double bond exocyclic to ring C. This compound is either inactive or demonstrates low activity in different systems. Due to its inactivity, $\Delta^{9,11}$ -THC has been recommended as a control in cannabinoid binding assays (3) as well as for studies dealing with *in vivo* distribution of cannabinoids.



Δ^9-THC



Δ^8-THC



$\Delta^{9,11}$-THC

Of the three isomers, Δ^8-THC is thermodynamically the most stable. Indeed in the presence of acids both Δ^9 - and $\Delta^{9,11}$-THC isomerize to Δ^8 -THC (4,5). Because of this difference in stability Δ^8-THC is produced as a by product during the synthesis of the other

two isomers and is found as a regular contaminant in Δ^8- and $\Delta^{9,11}$ -THC preparations. Also, $\Delta^{9,11}$-THC is obtained as a minor by-product during the preparation of Δ^9 -THC from Δ^8-isomer (6).

Traditionally, the tetrahydrocannabinol double bond isomers have been purified using column chromatography on Florisil (7). However, the process is quite tedious and does not usually lead to full separation. On the other hand, gas chromatographic techniques provide an effective method for the determination of these compounds as mixtures and in biological systems. As for the use of high pressure liquid chromatography with cannabinoids, Razdan *et al.* (8) was able to separate the 11-hydroxy isomers of Δ^8 - and Δ^9-THC, only after they were chemically modified to their respective acetate esters. Also, Valentine *et al.* (9) separated Δ^9 -THC from cannabidiol, cannabinol and cannabichromene in a gradient elution HPLC using a silica gel column. However, these four molecules differ significantly in structure.

This report details how, by using 10 μm octadecylsilane bonded packing and a ternary solvent system of water/acetonitrile/tetrahydrofuran, the separation of the three closely related tetrahydrocannabinol isomers (Δ^8-, Δ^9-, and $\Delta^{9,11}$-THC) has been achieved. The method can be used for an effective separation of these compounds in both analytical and preparative scales. It should also find application for assaying tetrahydrocannabinols in biological systems.

MATERIALS

Apparatus

A Waters Associates Liquid Chromatographic system equipped with a Model 590 Programmable Solvent Delivery Module, a U6K injector and a recycling manifold was used. The UV detector was a Waters Associates Series 440 Absorbance detector. The chromatograms were recorded using a single pen, 10 mv, Houston Instrument Microscribe

Series 4500. The reverse-phase column used was a μBondapak C18 column (Waters Assoc. Part No. 27324, Serial No. P60441B16) packed with 10 μm, irregularly shaped particles and had dimensions of 30 cm x 3.9 mm i.d. In front of the analytical column, was used a guard column, hand-packed with 10 μm C18-Corasil packing having dimensions of 5 cm x 4.6 mm i.d. For preparative scale a 30 cm x 7.8 mm i.d. (Waters Assoc. Part No. 84176, Serial No. P62231A01) μBondapak C18 column was used.

Chemicals

Individual samples of Δ^9-THC, Δ^8 -THC and $\Delta^{9,11}$-THC were synthesized in our laboratory. The acetonitrile, tetrahydrofuran and water were of HPLC quality and purchased from Fisher Scientific Company. All solutions and samples involved in the liquid chromatograhic separation were filtered through 0.22 μm Millipore filters prior to use.

RESULTS AND DISCUSSION

Various combinations of a ternary solvent system, water/acetonitrile/tetrahydrofuran (H_2O/AcN/THF) were used to effectively separate the three isomers of interest. As figure 1 shows, Δ^9-THC was well separated from Δ^8-THC (α = 1.15) using a 50/25/25 volume ratio of H_2O/AcN/THF respectively. At a flow rate of 2.0 mL/min the capacity factors (k′) were found to be 27.1 for Δ^9-THC and 30.33 for Δ^8-THC. However, this solvent system was unsuitable for the mixture of three tetrahydrocannabinols where $\Delta^{9,11}$ -THC eluted in between the other two isomers without proper separation. However, we were able to improve the separation by changing the ratio of the two organic solvents and maintaining the initial water ratio. Using this mixture, $\Delta^{9,11}$ -THC elutes with each of the other two isomers depending on the acetonitrile/tetrahydrofuran ratio, and gives only one isomer in pure form. For example, Δ^9 -THC was separated from Δ^8- and $\Delta^{9,11}$-THCs by using a 50/20/30 volume

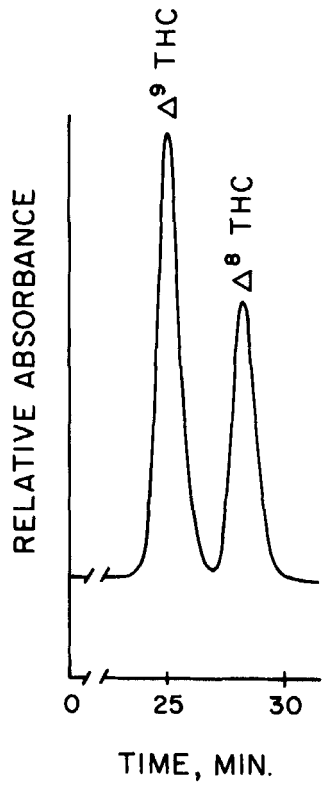


FIGURE 1. Isocratic Elution profile for a Δ^9-, Δ^8 -THC mixture. Conditions: column μBondapak C18, solvent H_2O/AcN/THF volume ratio of 50/25/25, flow rate 2.0 mL/min.

ratio of H_2 O/AcN/THF and Δ^8-THC could be obtained in pure form from the mixture by using a 50/30/20 volume ratio of H_2O/AcN/THF at a flow rate of 1.5 and 2.0 mL/min. respectively (Figure 2). Finally, optimal separation of these three isomers was achieved by using a 55/30/15 volume ratio of H_2O/AcN/THF respectively. At a flow rate of 1.5 mL/min a near base line separation was achieved for these compounds with k′ of 50.5, 53.5 and 57 for Δ^9-, $\Delta^{9,11}$ - and Δ^8-THC respectively and $\alpha_{12} = 1.06$ and $\alpha_{23} = 1.065$ (Figure 3). In order to achieve complete resolution of the isomers, we found it necessary to subject the mixture

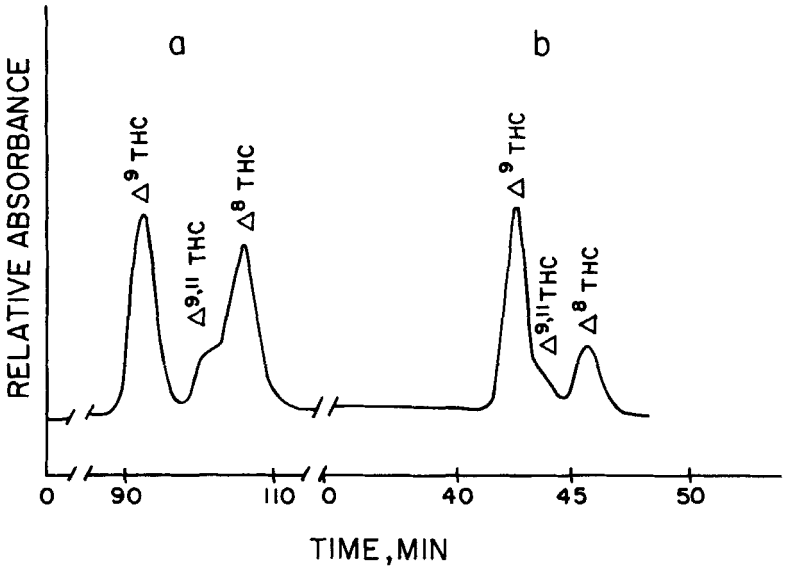


FIGURE 2. Isocratic Elution profile for a Δ^9-, $\Delta^{9,11}$ -, Δ^8-THC mixture using μ Bondapak C18 column and different H_2O/AcN/THF volume ratios of a) 50/20/30, flow rate 1.50 mL/min. b) 50/30/20, flow rate 2.0 mL/min.

to one recycle by means of recycle manifold. Following the above directions complete resolution was obtained after one recycle (Figure 3b).

We were able to apply the above analytical data for preparative separations. In a typical experiment, 100 mg of the sample containing the THC isomers, was dissolved in 2 ml of a H_2O/AcN/THF (40/35/25) mixture and eluted using the same ternary mixture used for the analytical separation (H_2O/AcN/THF; 55/30/15) and a flow rate of 4.0 mL/min. After one recycle the individual peaks were collected and the organic solvents were removed on a rotatory evaporator. The THC isomer was then extracted from the remaining aqueous using methylene chloride. The extracts were then dried over anhydrous Na_2 SO_4 and evaporated to give the pure THC isomers.

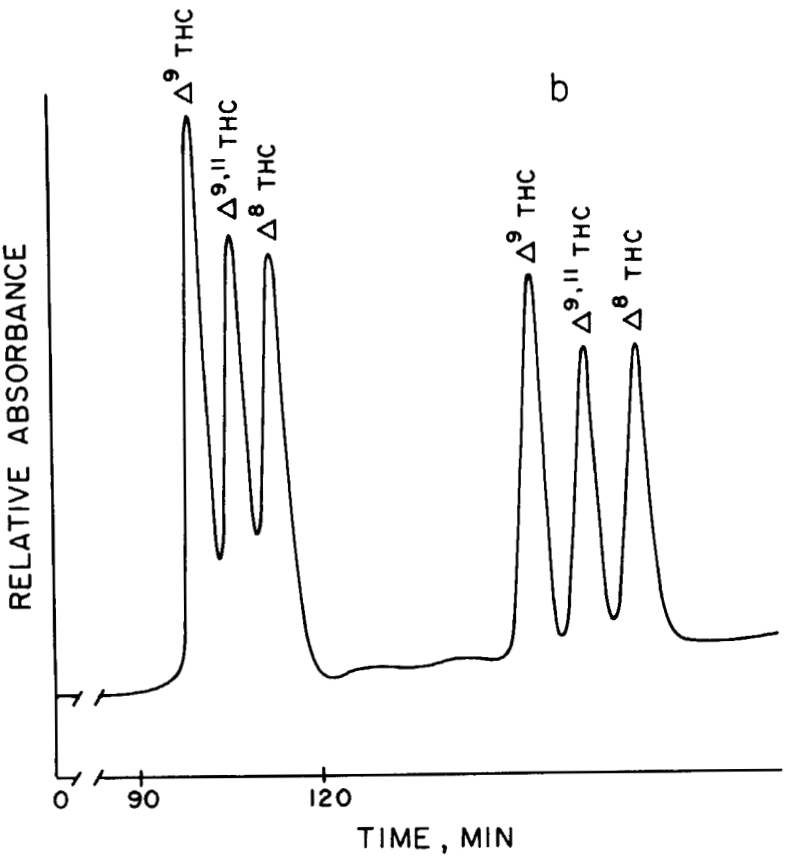


FIGURE 3. Isocratic Elution profile for a Δ^9 -, $\Delta^{9,11}$ -, Δ^8-THC mixture. Conditions : column μBondapak C18, solvent H_2 O/AcN/THF volume ratio of 55/30/15, flow rate 1.5 mL/min. b) After one recycle.

ACKNOWLEDGEMENTS

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