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The identification, isolation, and preservation of Δ^9-tetrahydrocannabinol (Δ^9-THC)*†

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(—)-*trans*-Δ^9-Tetrahydrocannabinol (Δ^9-THC) was isolated from marihuana plant extract, by adsorptive column and glc. The adsorptive column chromatography method consisted of chromatographing marihuana extract on a column packed with a mixture of silica gel (gas chromatography grade (100/120 mesh), silver nitrate and calcium sulphate ($CaSO_4 \cdot H_2O$) (3 : 1 : 0·5) with benzene as the eluting solvent. The glc method consisted of chromatographing the extract on a 3 ft silanized glass column ($\frac{3}{8}$ inch o.d.) packed with 1·5 ft of 2% QF-1 and 1·5 ft of 2% OV-17 on chromosorb W, AW 30–60 mesh, prep grade. A purity of 99% for the isolated Δ^9-THC was confirmed by infrared spectroscopy, nuclear magnetic resonance, mass spectroscopy. The effects of storage conditions on Δ^9-THC stability, monitored by glc, indicated the best method for preserving Δ^9-THC was at 0°, protected from light, stored under nitrogen.

Mechoulam & Gaoni (1967), Turk, Forney & others (1969) and most recently Mechoulam (1970) have reviewed adsorptive column chromatography techniques for the isolation of Δ-9-tetrahydrocannabinol (Δ^9-THC) and other cannabinoids from marihuana plant material.

Kingston & Kirk (1961), Farmilo & Davis (1961), Heaysman, Walker & Lewis (1967), Caddy, Fish & Wilson (1967), Lerner & Zeffert (1968), and Turk (1970) have reported methods for the separation and identification of marihuana components by gas liquid chromatography. However, methods for the separation and isolation of Δ^9-THC and other cannabinoids by preparative glc have not been found in the literature.

This paper reports two chromatographic methods for isolating Δ^9-THC and its stability under various storage conditions.

MATERIALS AND METHODS

Extraction of marihuana. Thailand marihuana was sieved through a 20 mesh screen and extracted in a Soxhlet apparatus with light petroleum (65–75°) for 3 h or until the glc analysis indicated the extraction of Δ^9-THC to be complete. The light petroleum extract was then washed with an aliquot of 4% NaOH in 50% ethanol (3 × 10 ml). The basic ethanolic fraction containing the cannabinoids was

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acidified to approximately pH 4 with 6N HCl and re-extracted with light petroleum (3 × 10 ml). This extract was flash evaporated under reduced pressure. The extracted resin was then dissolved in benzene for adsorptive column chromatography (3·2–3·5 g extract/5 ml benzene) or in chloroform for preparative glc.

Analytical assay of cannabinoids by gas liquid chromatography. The instrument was an F and M (Hewlett-Packard) Scientific 402 High Efficiency Gas Chromatograph, with a flame ionization detector and an L and N Speedomax W (0–1 mv) recorder having a chart speed of 30 inches/h. A single "U" shaped silanized glass column (4 ft by $\frac{1}{4}$ inch o.d.) was used; the first 1·5 ft were packed with 10% QF-1, the second 1·5 ft with a mixture of 1% OV-1, 1% OV-17 and the last 10 inches (nearest the detector side) with 2% OV-17. Each of these polymers were coated on chromosorb G, AW-DMCS, 100/120 mesh. The operating conditions were: injector port 285°; oven temp. 240°; flame detector 285°; carrier gas (He) flow 60 ml/min; hydrogen and oxygen flow rates 35 and 260 ml/min, respectively.

Isolation of $\triangle^9$*-THC by adsorptive column chromatography.* Silica gel (gas chrom. grade 100/120 mesh), silver nitrate and calcium sulphate ($CaSO_4 \cdot H_2O$) (250 g in a ratio of 3 : 1 : 0·5) (all materials were from Matheson, Coleman and Bell); were ground for 12 h, dried (85–90°) for approximately 2 h and 500 g of the hot adsorbent slurried with approximately 1 litre of benzene. The slurry was shaken until free of clumps and then transferred to a glass column (4·8 × 60 cm), which settled to a bed of approximately 4·8 × 45 cm. Benzene was passed through the column for 6 h, and then 3·2 to 3·5 g of marihuana resin per 5 ml benzene was placed on the column and eluted with benzene. Fractions (10 ml) were collected, concentrated by flash evaporation and the concentrate analysed by glc. All fractions containing Δ^9-THC, 99% pure, were pooled, washed three times with 0·2N HCl to remove the $AgNO_3$ and then stored under nitrogen at 0° protected from light.

Preparative glc isolation of Δ^9*-THC.* A Varian Aerograph Autoprep Model 712, equipped with an effluent splitter, collection system, and flame ionization detector was used. A single ovoid silanized glass column (3 ft by $\frac{3}{8}$ inch o.d.) was packed with 1·5 ft of 2% QF-1 and 1·5 ft of 2% OV-17 on chromosorb W, AW-DMCS 30–60 mesh. The operating conditions were: oven 230°; injector/detector 285°; exit tip 250°; carrier gas (He) flow 182 ml/min; hydrogen flow 33 ml/min; air flow 220 ml/min; and split ratio 10 to 1. Fig. 1 is a typical chromatogram produced by this system.

Decomposition of Δ^9*-THC stored under various conditions.* Δ^9-THC (pure by glc) was divided into six fractions. Each fraction was placed in a clear vial and stored under a different condition. The content of each vial was analysed periodically by glc, over five months, to determine the extent of decomposition. The storage conditions are listed in Table 2.

RESULTS AND DISCUSSION

Thailand marihuana after NaOH-ethanolic washing produced the gas chromatogram seen in Fig. 2. For an efficient separation of the cannabinoids, the ratio of Δ^9-THC to CBN must be at least 2 : 1. The marihuana used had a dry weight content of 4–6%, Δ^9-THC. Marihuana, having a Δ^9-THC content of 1% or less, does not yield pure Δ^9-THC in sufficient quantities to justify using this method.

Glc was primarily used to determine the components present in the eluted

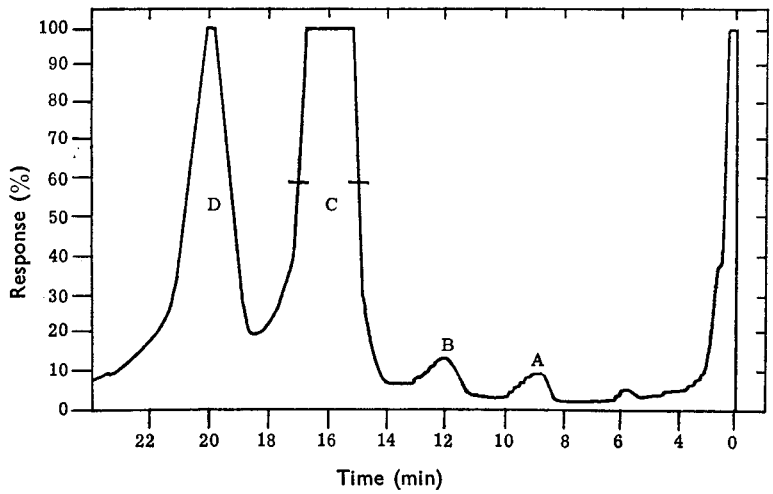

FIG. 1. Preparative gas chromatogram of marihuana extract and retention time of the cannabinoids. Column: 2% QF-1, 2% OV-17 on chromosorb W, AW 30/60 mesh. A. Cannabidiol. B. Δ^8-THC. C. Δ^9-THC. D. Cannabinol.

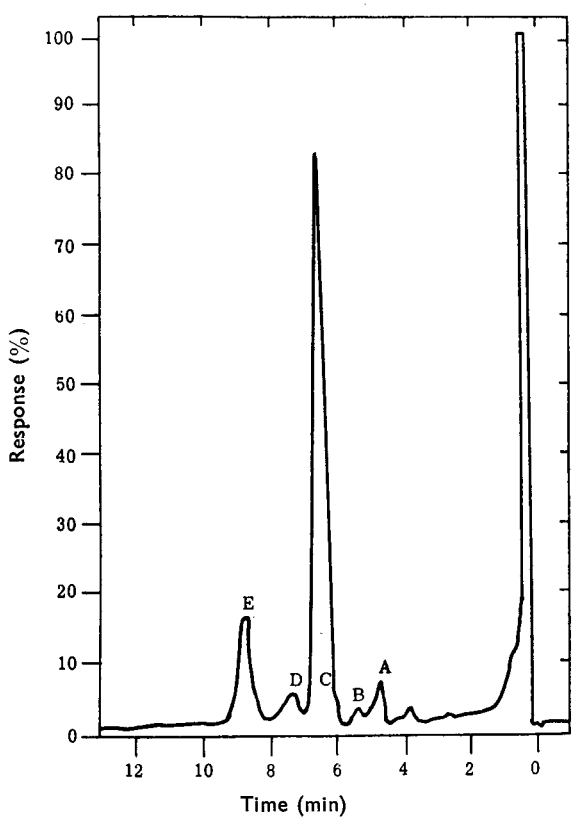

FIG. 2. Gas chromatogram of Thailand marihuana after NaOH-ethanolic washing. Column: 4 ft silanized glass column packed with 1·5 ft of 10% QF-1, 1·5 ft of a mixture of 1% OV-17, 1% OV-1, and the last 10 inches (nearest the detector side) with 2% OV-17. All polymers coated on chromosorb G, AW-DMCS, 100/120 mesh. A. Cannabidiol. B. Δ^8-THC. C. Δ^9-THC. D. Unknown. E. Cannabinol.

adsorptive column fractions. Δ^9-THC and Δ^8-THC can be separated using this analytical system (Fig. 3).

Pure cannabinol starts to be eluted at approximately 600 ml and ceases to be eluted after 800 ml; for Δ^9-THC the volumes are 900 ml to 1200 ml.

Adsorptive column chromatography. Purity of the Δ^9-THC isolated by this method was established by comparison of the infrared spectrum of Δ^9-THC with that for Δ^9-THC reported by Mechoulam & Gaoni (1967), by comparison of the nuclear magnetic resonance spectrum of the material with the known structure of Δ^9-THC

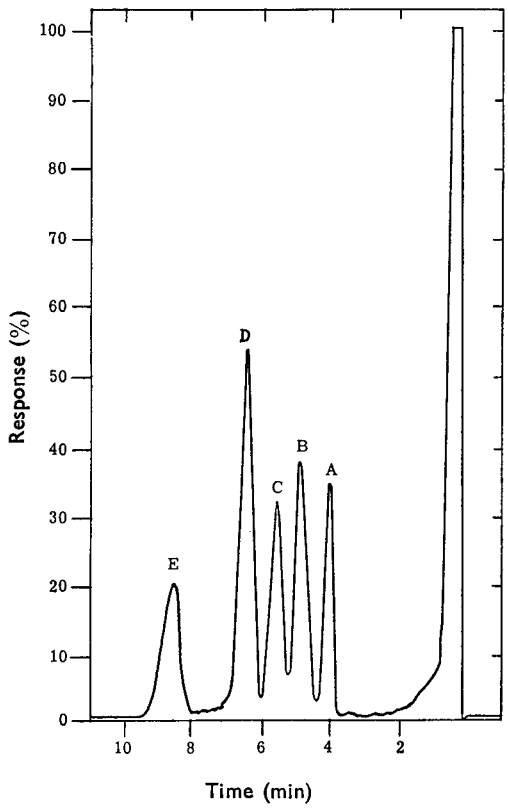

FIG. 3. Cannabinoids which can be separated, and detected in marihuana by gas liquid chromatography analysis. A. Cannabicyclol. B. Cannabidiol. C. Δ^8-THC. D. Δ^9-THC. E. Cannabinol.

(Mechoulam & Gaoni, 1967) and by mass spectrometry of the material which showed the isolated component was compatible with the spectrum reported for Δ^9-THC by Claussen, Fehlhaber & Korte (1969).

Pure cannabinol was also isolated by the adsorptive column chromatography method.

The efficiency of the method can be seen in Table 1. The Thailand marihuana used contained 4% (w/w) of Δ^9-THC. Recovery was 55%.

The amount of carboxylic precursors of THC isomers in the sample was not determined.

Table 1. *Recovery (%) of Δ⁹-Tetrahydrocannabinol (Δ⁹-THC)*

Sample	Weight of △⁹-THC in sample (g)	%△⁹-THC in sample (w/w)	% of Step I
Manicured Thailand marihuana (60 g) ..	2·40	4·0	—
Step I Solvent extraction (6·4 g Resin)	2·40	37·5	100
Step II NaOH-ethanolic washing (3·2 g Resin) ..	2·20	68·7	91·7
Step III Yield of △⁹-THC after adsorptive column chromatography (1·325 g △⁹-THC) ..	1·32	99·0	55

Preparative glc isolation of Δ⁹-THC. The advantage of preparative glc purification is that a compound can be isolated, collected, and stored under inert gas in one operation. In addition, more than one component can be collected if sufficient is present (see Fig. 1).

Purity of the isolated Δ⁹-THC was confirmed by infrared spectroscopy. The yield was 9 mg/24 h.

The Δ⁹-THC obtained by column chromatography was of equivalent purity but the amount of Δ⁹-THC that can be purified by the two methods differs widely. In 48 h (the time taken to elute Δ⁹-THC from the absorptive column) the average yield of 99% Δ⁹-THC from an adsorptive column was 1·2 g, whereas, with glc the amount was 18 mg.

Decomposition of purified Δ⁹-THC stored under various conditions. Table 2 shows the effects of storage conditions on the stability of Δ⁹-THC from marihuana. The findings were that the major decomposition product was cannabinol. Δ⁹-THC stored in acetone at room conditions decomposed at a more rapid rate than did the material stored under N_2 or exposed to air at room conditions. Decomposition was significantly inhibited by storage under nitrogen, air, or in acetone at 0° in the dark.

Table 2. *Decomposition (%) of purified Δ⁹-THC stored under various conditions*

		Room temperature, exposed to light			Refrigerated (0°), in dark		
Time analysed		Under N_2	Under air	In acetone	Under N_2	Under air	In acetone
Initial analysis	THC	100	100	100	100	100	100
	CBN	—	—	—	—	—	—
19th day	THC	100	100	96·7	100	100	100
	CBN	—	—	3·3	—	—	—
2nd month	THC	95·1	97·4	91·4	100	100	100
	CBN	4·9	2·6	8·6	—	—	—
4th month	THC	64·1	71·4	11·1	100	96·9	100
	CBN	35·9	28·6	88·9	—	3·1	—
5th month	THC	28·4	5·9	2·4	97·9	97·1	98·0
	CBN	71·6	94·1	97·6	2·0	2·9	2·0

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