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HIGH-PRESSURE LIQUID CHROMATOGRAPHY OF CANNABIS

IDENTIFICATION OF SEPARATED CONSTITUENTS

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SUMMARY

Δ^9-and Δ^8-Tetrahydrocannabinol, Δ^9-tetrahydrocannabinolic acid, cannabidiol, cannabidiolic acid, cannabinol, cannabinolic acid, cannabichromene and cannabichromenic acid were located in the liquid chromatogram of cannabis. Identifications were confirmed by gas chromatography–mass spectrometry.

INTRODUCTION

The use of high-pressure liquid chromatography (HPLC) for the comparative analysis of cannabis samples has been reported[1]. This paper describes the identification of cannabis constituents that can be separated by HPLC.

EXPERIMENTAL

Most of the work was carried out on a single block of cannabis resin in which all the major constituents normally observed by HPLC were present in reasonable quantity.

The methods used for HPLC, gas–liquid chromatography (GLC) and thin-layer chromatography (TLC) were essentially those described previously[1]. A Cecil 212 variable-wavelength UV detector was used for HPLC instead of the fixed-wavelength detector originally used. The same HPLC column was used for both analytical- and preparative-scale separations. Samples for preparative-scale HPLC were emulsified in an ultrasonic bath with a small volume of the eluting solvent and 50–100-μl aliquots were injected on to the column. Fractions were collected manually.

Samples were silylated with a 4:1 mixture of N,O-bis(trimethylsilyl)acetamide and trimethylchlorosilane.

Gas chromatography–mass spectrometry (GC–MS) was carried out with a Varian Aerograph Series 2700 gas chromatograph equipped with a 50:50 eluent splitter and coupled via a glass jet separator to a V.G. Micromass 12F single-focussing mass spectrometer. A 2-m × 0.4-cm glass column was used packed with 3% OV-17 on 80–100 mesh Gas-Chrom Q. Helium carrier gas, a flame ionisation detector and oven

temperatures of 230° or 250° were used. The accelerating voltages were 3 kV or 4 kV, the emission current was 100 μA, the electron energy was 70 eV and the source temperature was 240°. The scan time was 3 sec and the resolution of the spectra was 600–800 (10% valley). The mass spectra were uncorrected and were identified, where possible, by comparison with published data[2–9] or, in the case of some silylated compounds for which there were no published data, by comparing the spectra obtained with those expected on the assumption that the trimethylsilyl (TMS) groups increased the masses of all the major fragments equally without affecting the fragmentation patterns of the molecules. GLC retention data[10–13] and the molecular weights of the known naturally occurring cannabinoids[14] were taken into account when interpreting the mass spectra so that unequivocal identifications were obtained.

HPLC, GLC and GC–MS data are given in Table I.

TABLE I
HPLC, GLC AND GC–MS DATA

*Compound**	*Mol. wt.*	*HPLC relative retention time*	*GLC relative retention time*		*GC–MS data; m/e values of principle mass fragments (relative abundance as % of base peak)*
Δ^9-THC	314	100	100	—	314 (76), 299 (100), 271 (53), 258 (32), 246 (14), 243 (33), 232 (18), 231 (90), 193 (14)
Δ^9-THC monosilylated	386	—	—	100	386 (100), 371 (80), 343 (25), 330 (15), 315 (45), 304 (10), 303 (40), 265 (5)
Δ^9-THCA	—	203	—	—	—
Δ^9-THCA disilylated	502	—	—	296	502 (9), 487 (100), 431 (3), 419 (17)
Δ^6-THC	—	105	90	—	—
Δ^6-THC monosilylated	—	—	—	93	—
CBD	314	60	71	—	314 (9), 299 (6), 271 (5), 267 (9), 246 (19), 232 (18), 231 (100), 193 (7), 174 (14)
CBD disilylated	458	—	—	50	458 (11), 443 (6), 390 (100), 375 (6), 337 (26)
CBDA	—	69	—	—	—
CBDA trisilylated	574	—	—	136	506 (5), 491 (100), 453 (9)**
CBN	310	88	127	—	310 (13), 295 (100), 239 (5), 238 (18)
CBN monosilylated	382	—	—	138	382 (14), 367 (100), 311 (2), 310 (7)
CBNA	—	177	—	—	—
CBNA disilylated	498	—	—	375	498 (trace), 483 (100)
CBCh	314	131	70	—	314 (4), 299 (4), 232 (17), 231 (100), 174 (12)
CBChA	—	246	—	—	—
CBChA disilylated	502	—	—	278	502 (3), 487 (22), 420 (43), 419 (100)

* Abbreviations are explained in the text.
** Mass range insufficient to cover higher mass fragments.

RESULTS AND DISCUSSION

Preparative-scale fractionation

500 mg of cannabis resin were fractionated by HPLC. Fractions (Fig. 1) were collected and dilute ammonium hydroxide was added to neutralise the acid in the eluent. The fractions were then evaporated to dryness under reduced pressure at

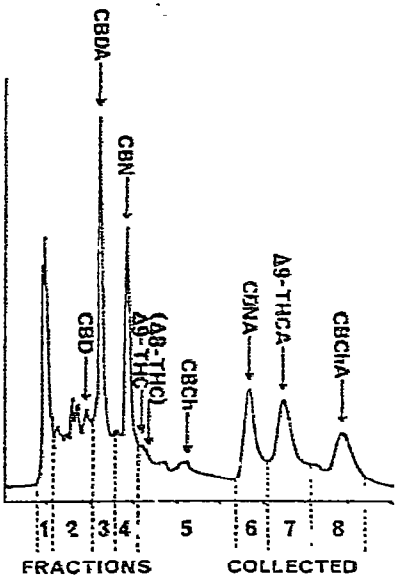

Fig. 1. HPLC of cannabis resin (100 mg extracted with 1 ml of eluting solvent) illustrating fractions collected and identity of peaks. 1 μl injected on to column. Detector wavelength, 254 nm; absorbance, 0.1. Scale graduations represent 0.5-min intervals. Abbreviations are explained in the text.

about 50°. The residues thus obtained were extracted with methanol, filtered through glass wool and evaporated to small volume. A portion of each fraction was examined by HPLC which showed that fractionation had occurred although there was a considerable overlap between some of the fractions. TLC and GLC revealed no cannabinoids in Fraction 1, but the other fractions contained mixtures of cannabinoids suggesting that, in addition to being heterogeneous, the fractions had probably undergone decomposition (such as partial decarboxylation of acidic cannabinoids) during the extraction procedure.

Identification of neutral cannabinoids and effect of varying detector wavelength

Cannabinol (CBN), cannabidiol (CBD), Δ^8- and Δ^9-tetrahydrocannabinol (Δ^8- and Δ^9-THC) were located in the liquid chromatogram by running pure standards. UV absorption data[14] indicated that the sensitivity of the HPLC detector would be greatly affected by the wavelength chosen for monitoring the eluent. In practice, it was found that the four standard cannabinoids gave a good response at low wavelengths (210–220 nm) whereas only CBN gave a reasonable response at longer wavelengths (240–310 nm). The use of a 254-nm fixed-wavelength UV detector for the quantitative estimation of Δ^9-THC by HPLC[15] is therefore a relatively insensitive procedure. However, examination of cannabis resin by HPLC with detector wavelengths varying between 210 and 450 nm showed that a maximum number of components was detected at 250–260 nm, thus the use of a 254-nm detector is to be recommended for comparative cannabis analysis[1]. Liquid chromatograms run at 220, 254 and 280 nm are shown in Figs. 1 and 2.

The locations of CBN, CBD and Δ^9-THC in the liquid chromatogram of cannabis resin were confirmed by comparing the responses at different wavelengths of the

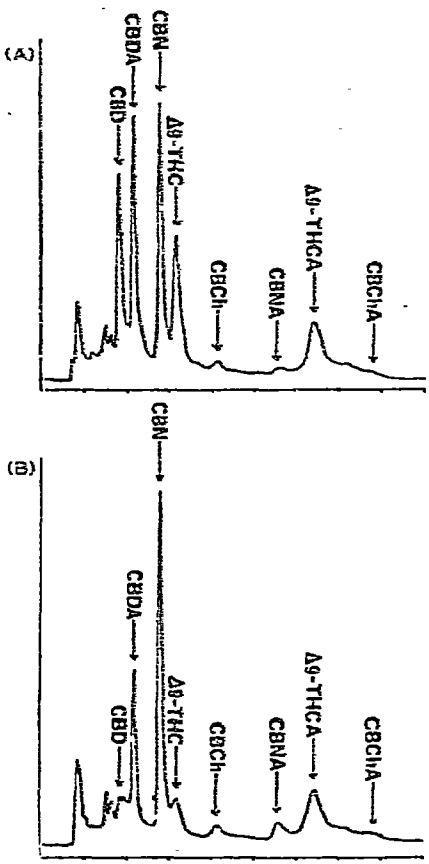

Fig. 2. HPLC of cannabis resin (100 mg extracted with 1 ml of eluting solvent) illustrating effect of different detector wavelengths. 1 μl injected on to column. (A) Detector wavelength, 220 nm; absorbance, 0.5. (B) Detector wavelength, 280 nm; absorbance, 0.2. Scale graduations represent 0.5-min intervals. Abbreviations are explained in the text.

suspected peaks with those of the pure standards. Further evidence was obtained by peak enhancement on the addition of pure standards to the cannabis resin extracts used for HPLC; GLC examination of the preparative-scale fractions (see previous section) confirmed the presence of CBN, CBD and Δ^9-THC in the relevant portions of the liquid chromatogram. In addition, the removal of acidic cannabinoids from the cannabis resin by alkaline extraction[14] prior to HPLC did not affect the CBN, CBD or Δ^9-THC peaks. Little, if any, Δ^8-THC was found in the resin by HPLC and GLC, but the presence of CBN, CBD and Δ^9-THC in the resin was confirmed by GC–MS.

Preparation of an acidic cannabinoid fraction and identification of acidic cannabinoids

A light petroleum (boiling range 40–60°) extract of 500 mg of cannabis resin was filtered and extracted twice with 1 *N* potassium hydroxide. The combined potassium hydroxide extracts were back-extracted twice with light petroleum, acidified with 2 *N* sulfuric acid and extracted three times with light petroleum. The extraction sequence was then repeated and the final solution of acidic cannabinoids in light

petroleum was dried with anhydrous sodium sulfate and evaporated at low temperature to small volume. HPLC revealed four major components (Fig. 3) corresponding to Fractions 3, 6, 7 and 8 (Fig. 1). A portion of the acidic mixture was evaporated to dryness, decarboxylated[14] at 100° under nitrogen for 1 h and examined by HPLC. The major decarboxylation products were CBN, CBD and Δ^9-THC, showing that cannabinolic acid (CBNA), cannabidiolic acid (CBDA) and Δ^9-tetrahydrocannabinolic acid (Δ^9-THCA) were present in the acidic mixture. GLC examination of the acidic mixture gave similar results since acidic cannabinoids are decarboxylated in the heated injection port of a gas chromatograph[14]. A portion of the acidic mixture was silylated to prevent decarboxylation[14] and examined by GLC and GC–MS. Three major components were obtained and identified as the TMS derivatives of CBNA, CBDA and Δ^9-THCA. CBNA was present in much smaller quantity than CBDA and Δ^9-THCA. GC–MS cannot adequately distinguish between the two known isomers of Δ^9-THCA in the absence of authentic standards, but it is likely that the isomer encountered in this study was Δ^9-THC acid A since this is a major acidic constituent of cannabis[11,14].

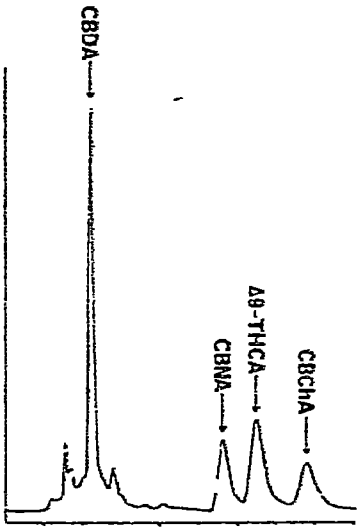

Fig. 3. HPLC of acidic cannabinoid fraction (arbitrary concentration). Detector wavelength, 254 nm. Scale graduations represent 0.5-min intervals. Abbreviations are explained in the text.

Fractions 3 and 6 (Fig. 1) obtained by preparative-scale fractionation of the cannabis resin each contained one major acidic component. Alkaline extraction followed by acidification and back-extraction into light petroleum were used to isolate these acids and virtually homogeneous products were obtained. Decarboxylation of the acid from Fraction 3 gave CBD as the major product on GLC and HPLC, showing that the acid was CBDA. This was confirmed by GLC of the silylated acid when the TMS derivative of CBDA was identified. The acid from Fraction 6 was analysed similarly and identified as CBNA.

Fractions 7 and 8, which contained the remaining two acidic cannabinoids, were heterogeneous, but relatively homogeneous products (Fractions 7A and 8A) were obtained by preparative HPLC of the remainder of the acidic cannabinoid mixture.

Fraction 7A was then analysed in the same manner as Fractions 3 and 6 and identified as Δ^9-THCA. The acid in Fraction 8A was present in insufficient quantity for detailed analysis, but its decarboxylation product was examined by HPLC and found to correspond to a minor, unidentified component of the original cannabis resin. This component was isolated by preparative HPLC from a resin sample in which it was present in reasonable quantity and identified by GC–MS as cannabichromene (CBCh), indicating that the acid in Fraction 8A was cannabichromenic acid (CBChA). This was confirmed by extracting the suspected CBChA from 2 g of the original cannabis resin sample by preparative HPLC, when sufficient material was obtained to identify CBChA and its decarboxylation product, CBCh, by GC–MS. The GLC retention data showed that cyclization of CBCh to cannabicyclol[7] as a result of the decarboxylation process did not occur. CBChA was shown by GLC to be a minor component of the cannabis resin sample, therefore its appearance as a major peak on HPLC can be attributed to a high extinction coefficient at 254 nm.

The results show that all the major and some of the minor cannabinoids normally present in cannabis resin can be detected by HPLC, a degree of selectivity being conferred on the method by an appropriate choice of detector wavelength. A useful feature is the excellent separation of CBD and CBCh, which cannot be achieved by GLC without silylation[12]. Both acidic and neutral cannabinoids are readily detected by HPLC, which explains the superiority of HPLC over GLC as a technique for comparative cannabis analysis[1]. In addition, HPLC offers a means of determining acidic and neutral cannabinoids quantitatively without having to esterify or silylate the samples to prevent decarboxylation, and a study with this aim is in progress.

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