J. Pharm. Pharmac., 1973, **25**, 150–155 *Received August* 31, 1972

The extraction and estimation of the cannabinoids in *Cannabis sativa* L. and its products

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A convenient method for the complete extraction of the cannabinoids from fresh plant material, herbal cannabis, cannabis resin and reefers, has been devised. Chloroform was a more suitable solvent than light petroleum or ethanol and simple shaking of powdered material with the solvent was effective. Fresh material should be air-dried and powdered before extraction. The main cannabinoids in the extract are determined by g.l.c. using androst-4-ene-3,17-dione as internal standard. The coefficient of variation for repeated determinations of THC on a single extract was 1·4%; for all operations, including sampling and extraction, it was 2·7%. Duplicate analyses of 24 samples of herbal cannabis and of 20 reefers, all of varying potency, showed that the errors fell within the expected limits for THC, CBD and CBN. The method is simple and rapid; duplicate determinations can be completed in about 2½ h.

A convenient and reproducible method of extracting and estimating the important cannabinoids in the cannabis plant and its products is needed. For the estimation gas-liquid chromatography is preferable, and we have found the method of Lerner (1969) as modified by Fetterman, Keith & others (1971a) satisfactory. However, the *extraction* procedures published vary and evidence on their precision and reliability is seldom given. Several treatments such as grinding (Toffoli, Avuco & others, 1968; Ohlsson, Abou-Chaar & others, 1971), heating (Kimura & Okamoto, 1970), repeated blending (Chung Hun Song, Kanter & Hollister, 1970), percolation (Davis, Farmilo & Osadchuck, 1963) and prolonged soaking (Aramaki, Tomiyasu & others, 1968) have been used; the solvents recommended also varied and include light petroleum, ethanol and chloroform; Claussen & Korte (1968) also used dichloromethane. Concentration or de-waxing of the extracts has also been advocated. Kimura & Okamoto (1970) recommend that the plant material should be heated before extraction to convert cannabinoid acids into more soluble form, but this suggestion is not supported by the results of Fetterman, Doorenbos & others (1971b). We have therefore examined various factors involved in the extraction and estimation of fresh and dried plant material, cannabis resin and reefers.

MATERIALS

Cannabis plants. One sample (SP2) was grown out-of-doors in our experimental garden in London from seeds originally obtained from Kathmandu, Nepal. The other (SP5) was from a police seizure of plants grown partly in a house and later in a greenhouse in London.

Cannabis resin. This was from a customs seizure in 1969 and was probably of Pakistan origin.

Reefers. Several reefers typical of those in current use in London were used (further details will be published).

METHODS

Extraction from fresh plant material

Since the active resin is claimed to occur in the superficial glandular trichomes, it should be possible to dissolve it out by prolonged immersion of unbroken fresh material in light petroleum with the extraction of a minimum of ballast substances. However, even after the material had been soaked in light petroleum for eight weeks only about 50% of the THC content was yielded to the solvent. Chemo-microscopical investigation of cannabis plants showed that, while most of the resin was in the glands, significant amounts occurred in non-glandular tissue (Fairbairn, 1972), which presumably is difficult to extract by soaking unbroken material. It was therefore decided to air-dry first, then powder, before extraction, and to use non-glandular material initially since if a satisfactory method could be devised for this it should be adequate for the more easily extracted superficial glands and for cannabis resin itself.

Extraction from air-dried non-glandular plant material

Fresh material (type SP2) with most of its main stems removed, dried fairly rapidly when spread out and left at room temperature (about 20–22°). After three days about 70% loss in weight occurred resulting in a brittle easily powdered product. No further loss occurred during the next four days. The air-dried material contained 8 to 9% moisture as determined by heating at 105° for 3 h. After being dried, all samples were powdered and stored in closed bottles in the dark, at room temperature, until required for use.

Various extraction procedures were used with light petroleum (60–80°) as solvent. The results showed that a single shaking extracted only 88–94% of the THC and a double shaking 94–99%. Further extraction with cold or boiling petrol only yielded traces of THC; more THC was, however, extracted with ethanol. Unfortunately, significant amounts of pigment and ballast were also extracted by the ethanol and these interfered with the gas chromatography.

The results obtained using chloroform as a solvent (Table 1) show that a single extraction yielded 98–99% THC and a double extraction almost 100%. Once more,

Table 1. *Analyses of various chloroform extracts of non-glandular leaf material (SP2).* Results expressed as mg THC g^{-1} air-dried leaf.

Conditions of extraction	Results		
1(*a*) 50 mg shaken ½ h with 3 ml $CHCl_3$, filtered; filter washed till volume 5 ml .. ..15·45;	16·45;	16·33	Mean 16·08
1(*b*) Residue from 1(*a*) treated with $CHCl_3$ as above .. 0·37;	0·17;	0·13	Mean 0·22
Total extracted ..			16·30
2(*a*) 50 mg shaken ½ h with 3 ml $CHCl_3$, filtered; residue re-extracted by shaking with $CHCl_3$ to 10 ml ..16·05;	16·60;	15·65	Mean 16·10
2(*b*) Residue from 2(*a*) extracted with boiling $CHCl_3$ .. 0·06;	Nil	Nil	Mean 0·02
2(*c*) Residue from 2(*b*) re-extracted with boiling $CHCl_3$ Nil	Nil	Nil	
2(*d*) Residue from 2(*c*) extracted with 3 ml ethanol, etc... 0·19;	0·12;	0·05	Mean 0·12
Total extracted:..			16·24

a small amount was extracted from the chloroform exhausted residue using ethanol, but much more pigment and ballast was extracted than with chloroform.

Extraction of gland-containing tissue, cannabis resin and reefers

Leaf material. (SP5) with abundant sessile glands was extracted by the recommended method (p. 153) to yield an average of 8·47 mg THC g^{-1}: further extraction with ethanol gave 0·10 mg THC g^{-1} indicating that 99% was extracted by the recommended method. Since the glands in the residue still seemed intact on microscopical examination, the residue was ground with ethanol but no further yield of THC was obtained.

Surprisingly *cannabis resin* could also be extracted by simple shaking; the chloroform rapidly penetrated the soft mass which broke up into fragments. There were difficulties in filtration, however, because a felt of trichomes and other vegetable debris blocked the sintered glass or filter paper. The only satisfactory method was to centrifuge and decant. The results given in Table 2 show, once more, that a double shaking with chloroform yields more than 99% of the cannabinoids: further grinding with chloroform or with ethanol only produces a fraction more.

The *reefers* were all mixtures of tobacco with either powdered resin or herbal cannabis. Therefore, tobacco alone was extracted by the standard method: gas-chromatography showed the absence of significant amounts of components with retention times similar to those of the cannabinoids. Since the absolute amount of cannabinoids per reefer is normally required, they were extracted *in toto* without

Table 2. *Results of analyses of one batch of cannabis resin using chloroform as a solvent*

Sample		Conditions of extractions	Cannabinoids mg g^{-1} THC (*a*)	CBN(*b*)	CBD(*c*)	Totals
1	(*a*)	50 mg shaken ½ h with solvent: filtered, washed with solvent to volume	40·6	37·0	56·5	
	(*b*)	Residue from 1(*a*) shaken ½ h with solvent; washed, etc.	0·28	Nil	0·62	
	(*c*)	Residue from 1(*b*) ground with solvent	0·16	Nil	0·19	
		Totals:	41·04	37·0	57·31	135
2	(*a*)	As for 1(*a*)	61·4	29·4	59·0	
	(*b*)	As for 1(*b*)	0·26	Nil	1·25	
	(*c*)	As for 1(*c*)	0·10	Nil	0·25	
		Totals:	61·76	29·4	60·50	152
3	(*a*)	As for 1(*a*)	50·9	30·1	56·1	
	(*b*)	As for 1(*b*)	0·29	Nil	0·48	
	(*c*)	As for 1(*c*)	0·58	Nil	0·98	
		Totals:	51·77	30·1	57·56	139
4	(*a*)	50 mg shaken twice as in recommended method	23·1	46·4	58·6	
	(*b*)	Residue from 4(*a*) extracted with ethanol	0·65	0·20	0·47	
	(*c*)	Residue from 4(*b*) ground with ethanol	Nil	Nil	Nil	
		Totals:	23·75	46·60	59·07	129
5	(*a*)	As in 4(*a*)	46·5	36·2	59·8	
	(*b*)	As in 4(*b*)	0·20	0·15	0·49	
	(*c*)	As in 4(*c*)	Nil	Nil	Nil	
		Totals:	46·70	36·35	60·29	143

(*a*) THC = tetrahydrocannabinol.
(*b*) CBN = cannabinol.
(*c*) CBD = cannabidiol.

previous powdering. Each reefer was triturated with the solvent and filtered (without difficulty). More than 99% of the cannabinoids were extracted by the recommended method.

Gas-chromatography

The gas chromatography procedure is basically that of Lerner (1969) as modified by Fetterman & others (1971a). A Pye 104 instrument with flame ionization detector was used. The column was 5 ft in length and 4 mm internal diameter packed with 2% OV17 on Chromosorb W (AW-DMCS, 80–100 mesh) and operated at 235–240° with a nitrogen flow rate of 45 ml min^{-1}. Peak area was estimated by measurement of height × (width at half height). The internal standard androst-4-ene-3,17-dione was calibrated against Δ^1-tetrahydrocannabinol (THC), cannabinol (CBN) and cannabidiol (CBD) which had retention times (internal standard =1·0) as follows: CBD 0·37, Δ^1-THC 0·51 and CBN 0·64. The ratio of the area of the peaks per unit weight for CBD: THC: CBN was 104:93:84.

Statistical analysis

Repeated determinations on a single mixture of Δ^1-THC and internal standard gave results for the concentration of THC with a coefficient of variation of 1·4%. The leaf material SP2 was assayed 11 times by the recommended method, one gas chromatographic determination only being made from each 50 mg of powdered leaf. The mean of the 11 results was 14·59 mg THC g^{-1} with a standard deviation of 0·389 and coefficient of variation of 2·7%.

A further 24 samples of herbal cannabis, prepared from different plants and varying in THC content from about 8 to 33 mg g^{-1}, were analysed in duplicate (two separate weighings). An estimate of the coefficient of variation, calculated from the following formula gave a value of 2·55%.

$$\text{Coefficient of variation} = \frac{100}{\sqrt{N}} \sqrt{\sum \left(\frac{x_2 - x_1}{x_2 + x_1}\right)^2}$$

Where N = number of pairs of assays and x_2 and x_1 = results from each pair. Similar estimates for the CBD content, when present in more than trace amounts, gave estimates for the coefficient of variation of 3·40% (9 pairs of results).

The results from duplicate analysis of single extracts from 20 reefers gave estimates for the coefficient of variation for THC of 2·23% (twenty pairs varying from 0·5 to 29 mg THC per reefer), for CBD of 2·84% (19 pairs varying from 0·1 to 38 mg CBD per reefer) and for CBN of 3·96% (18 pairs varying from 0·3 to 21 mg CBN per reefer).

Recommended method

Reduce freshly collected material, dried by exposure to a current of air at 20–22° for three days, or herbal cannabis, used unchanged, to a sufficiently fine powder to pass through a No. 44 sieve (355 μm aperture). Store in a closed bottle in a cool place in the dark until required. Shake about 50 mg of the powder, accurately weighed, with chloroform (3 ml) in a stoppered tube for ½ h; filter through a sintered glass disc (porosity 3, diameter 10 mm) and wash the filter with two small portions of solvent. Transfer the residue back to the stoppered tube and re-extract with a further 3 ml of

chloroform as before. Combine the filtrates and make up to 10·0 ml. Mix 1 ml of the filtrate with a suitable volume of chloroform solution of the internal standard, androst-4-ene-3,17-dione (about 0·1 mg ml^{-1}) and use 2 to 8 μl of the mixture for gas chromatography. For cannabis resin, follow the same procedure but centrifuge the extracts instead of filtering. For reefers, triturate the entire contents, without previous powdering, with successive amounts of solvent, filter and make up to 10–20 ml according to the original weight which may vary from $\frac{1}{4}$ to 1 g.

DISCUSSION

With fresh plant material it was not possible to extract more than about 50% of the cannabinoids by prolonged soaking (56 days) of unbroken material in solvent. Contributing factors might be the presence of some of the cannabinoids in the internal tissues and complexing with components of the intact glands. It is not due to the insolubility of the cannabinoid acids in organic solvents, as suggested by Kimura & Okamoto (1970), since large amounts of these acids were found in our extracts, using thin-layer chromatography (de Faubert Maunder, 1969; Mechoulam, Ben-Zvi & others, 1969). Air-drying of fresh plant material followed by powdering was found to be much more satisfactory.

Although light petroleum is frequently recommended as a solvent, we found it to be less efficient than chloroform. The experiments with chloroform indicated that quantitative extraction was achieved for both glandular and non-glandular leaf, as further vigorous treatment of the exhausted residue did not yield significant amounts of cannabinoids. Fetterman, Doorenbos & others (1971b) also showed that only minute amounts of cannabinoids can be extracted with ethanol, from chloroform-exhausted material. But ethanol is not a suitable solvent because it also extracts ballast substances.

The method had a satisfactory reproducibility. Using a single mixture of THC and internal standard the coefficient of variation (c.v.) for the g.l.c. operation was 1·4% compared with the value of 1·1% found by Lerner (1969) also using a single mixture. For a single sample of powdered herb the total errors involved in extraction and g.l.c. corresponded to a c.v. for the THC of 2·7%. Since CBD and CBN were present only in trace amounts it was not possible to determine c.v. The c.v. of the THC results was confirmed and estimates of the c.v. for CBD were obtained from the results from duplicate determinations on 24 samples of herb which varied widely in their cannabinoid content. Estimates of the c.v. were: for THC content, 2·55%; for CBD, 3·40%. The method extracted the cannabinoids completely from cannabis resin, alone or in admixture with tobacco (which did not interfere significantly with g.l.c.) since each reefer is only extracted once, replicate analyses involving extraction errors cannot be made, however 20 reefers were analysed in duplicate (separate dilutions of extract and internal standard) giving estimates of the c.v. for THC of 2·23%, for CBD 2·84% and for CBN of 3·96%.

The values for THC confirm that the errors expected from the c.v., determined on one sample of herb, are not exceeded in the 24 other samples and 20 samples of reefer examined. The c.v. values are higher for CBD and CBN since the quantity present was usually much lower than that of THC and the areas of the peaks were low. For more accurate determinations, larger amount of extract could be used but the large peak for THC would then have to be ignored. The relative retention times we found for CBD, THC and CBN are similar to those calculated from Fetterman & others,

1971a (0·34, 0·51 and 0·63 respectively). We have also given the ratios of peak area per unit weight for the three cannabinoids to show that an instrument calibrated with pure THC could be used for determining the other components, though with less accuracy.

Our method is convenient and rapid; duplicate samples of material can be extracted and analysed in about 2½ h, requiring 1 h of the operator's time.

The analysis of cannabis resin presents a sampling problem (see Table 2). The sample was originally a slab of fairly soft material but, during storage, the outside darkened and hardened and would be deficient in THC owing to oxidation to cannabinol (CBN) (Mechoulam, 1970) so that the composition of the sample would vary. However, the *sum* of THC and CBN should be more constant as also the amount of cannabidiol (CBD), which seems quite stable. The total cannabinoids therefore should be reasonably constant and this can be seen from the results which show a variation from the mean of $\pm 8\%$ compared with that for THC of $\pm 42\%$. Since this type of resin may be difficult to reduce to fine powder, a larger sample than that used in the recommended method would be necessary with appropriately larger amounts of solvent. Samples of resin suitable for powdering can be treated by the normal method.

Separate determination of free cannabinoids and their corresponding acids is possible by the method of Fetterman & others (1971b). However, since the acids are converted on smoking to the free cannabinoids (Mechoulam, 1970) we recommend our more simple method by which the total cannabinoids available on smoking are determined. However, if the material is intended for oral use (thermally stable conditions) separate determinations of the acids and free cannabinoids should be made.

Acknowledgements

We would like to thank the Medical Research Council for a grant towards the cost of this work, Miss E. Sayron and Dr. S. Simic for some analytical assistance, Prof. L. Saunders for advice on mathematical treatment and Prof. R. Mechoulam for supplies of pure THC, CBD and CBN and Dr. P. N. Suwal for the sample of Nepalase seed.

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