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The tetrahydrocannabinol and tetrahydrocannabinolic acid content of cannabis products

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Cannabinoid acids readily decarboxylate to the corresponding cannabinoid. Methods are available for the determination of Δ^9-tetrahydrocannabinol (THC) and its acids (THCA) and published data on the levels of these compounds in cannabis are summarized. Using gas and liquid chromatography, fresh cannabis (64 samples) and cannabis resin (26 samples) from different countries were examined. Wide variations in the relative amounts of THCA and THC in cannabis were found. For cannabis resin, a wide range of values was also found (0·5 : 1 to 6·1 : 1), the lower values being in resins from the Indian sub-continent and the higher values in resins from the Mediterranean area. Total THC values were in the range 1·0–10·6% in cannabis and 6·0–12·5% in cannabis resin.

The principal physiologically active component of cannabis is Δ^9-tetrahydrocannabinol (THC). Cannabis also frequently contains Δ^9-tetrahydrocannabinolic acid (THCA 'A')† (Fetterman et al 1971; Turner & Hadley 1973). A second acid, designated THCA 'B'†, which is found at much lower concentrations, has been identified (Mechoulam et al 1969; Turner et al 1974). These acids readily decarboxylate to yield THC. The reaction occurs on storage (Claussen & Korte 1968; Masoud & Doorenbos 1973), under the influence of light (De Zeeuw et al 1972a,b) or on heating (Kimura & Okamoto 1970; De Zeeuw et al 1972a,b). Decarboxylation contributes to the greater activity of cannabis products when they are smoked compared with ingestion (Miras et al 1964; Mechoulam et al 1970; Razdan 1972). Therefore both the THC and THCA contents of cannabis products should be measured. Decarboxylation also takes place rapidly on injection of cannabis extracts into a gas chromatograph (Davis et al 1963; Claussen & Korte 1968; Toffoli et al 1968; Fetterman et al 1971; Masoud & Doorenbos 1973).

Three principal methods have been used to determine the content of acidic cannabinoids in cannabis and cannabis resin. The sample extracts may be separated into neutral and acidic fractions before analysis by gas liquid chromatography (g.l.c.) (Yamauchi et al 1967; Toffoli et al 1968; Masoud & Doorenbos 1973; Smith 1975). There may however be breakdown of acidic cannabinoids during preparation of the fractions. Direct g.l.c. analysis of mixtures containing both neutral and acidic cannabinoids may be carried out if silyl (Turner et al 1973; Knaus et al 1976), or other stable and volatile derivatives (Crombie 1976) are prepared. Quantitation is, however, not always reliable because of competing reactions from other constituents, and furthermore the use of silanizing reagents can be detrimental to the performance of the flame ionization detector.

High performance liquid chromatography (h.p.l.c.) will resolve simultaneously both acidic and neutral cannabinoids without prior chemical separation or derivative formation. Quantitation, in the absence of authentic standards of the acidic cannabinoids, is achieved by subjecting the sample simultaneously to g.l.c. and h.p.l.c. analysis (Smith & Vaughan 1976). G.l.c. analysis will give the 'total' THC present, i.e. the THC originally in the sample together with that formed by decarboxylation of THCA ('A' and 'B') upon injection (Baker et al 1980a). H.p.l.c. analysis will give the amount of THC originally in the sample. Hence the THCA content may be calculated.

Despite the availability of methods for the quantitative determination of acidic cannabinoids, few studies have been made on samples of known origin. Furthermore, of those examined, few were fresh and any acids present may have undergone considerable decarboxylation during storage. Table 1 summarizes the published data on amounts of THC and THCA found in cannabis products of known origin. It should be noted that most of these data relate to cannabis grown in the U.S.A., albeit from seeds from other countries. The aim of the present study is to generate information on the THC

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† systematic names are 6a,7,8,10a-tetrahydro-1-hydroxy-6,6,9-trimethyl-3-pentyl-6*H*-benzo[*c*] chromene-2-carboxylic acid (for THCA 'A') and -4-carboxylic acid (for THCA 'B').

Table 1. Summary of published data on THCA and THC contents in cannabis products of known origin. All samples are cannabis unless otherwise stated.

Country of Origin	Age (months)	THCA (% w/w)	THC (% w/w)	Reference
Mexico*	Fresh	1·2	0·1	1
	6	1·0	0·22	2
	6	1·0	0·16	2
	6	0·71	0·45	2
	18	0·43	0·57	2
	18	0·1	0·5	2
	?	1·49	0·48	3
	6	1·4	0·07	4
	6	1·2	0·3	4
	18	0·29	0·55	4
USA Iowa	18	0·20	0·34	2
Iowa	18	trace	trace	2
Des Moines	18	trace	trace	2
Minnesota	18	trace	0·04	2
Minnesota	6	0·04	0·08	2
Minnesota	18	trace	0·03	2
Turkey*	6	0·06	0·01	2
	6	0·02	0·04	2
	?	0·05	0·12	3
Turkey (resin)	?	9·50	2·48	5
Sweden*	6	trace	trace	2
France*	6	trace	trace	2
Italy*	6	0·15	0·06	2
	6	0·13	0·05	2
Lebanon (resin)	10–15	3·1	1·4	6
(resin)	?	1·4	3·4	6

* grown in USA (Mississippi) from seed of stated origin.
[1] Turner et al 1973; [2] Masoud & Doorenbos 1973; [3] Kinzer et al 1974; [4] Fetterman et al 1971; [5] Verwey & Witte 1972; [6] Mechoulam 1970.

and THCA content of fresh cannabis products. Such information may, in conjunction with other chemical data, be of value in forensic, pharmacological and chemobotanic studies.

MATERIALS AND METHODS

Δ⁹-Tetrahydrocannabinol Standard

Δ^9-Tetrahydrocannabinol was purchased (Makor Chemicals Ltd, PO Box 6570, Jerusalem, Israel) as a solution (1 g in ethanol) which was diluted with ethanol (PBS grade) to 100 ml and stored in the dark at 0 °C. Standard solutions were prepared daily by diluting 1 ml of the stock solution to 50 ml (for g.l.c.) or 10 ml (for h.p.l.c.) with ethanol. 5 μl injections (1 μg THC) were made for g.l.c. and 2 μl injections (2 μg THC) for h.p.l.c.

Sample selection

Samples of cannabis and cannabis resin were taken from seizures made by Officers of HM Customs and Excise during 1979. The most probable country of origin was assigned by taking into account information from the carrier, physical appearance of the specimen (de Faubert Maunder 1976) and thin layer chromatographic characteristics (Baker et al 1980b). Only samples which, to the best of our knowledge, were fresh on arrival in the UK were included in this study. All samples were analysed within a month of seizure, during which time they were stored in sealed plastic bags at room temperature in the dark. Freshness was determined by the absence or near absence of cannabinol (CBN), this being determined by t.l.c. (de Faubert Maunder 1969). CBN is the major breakdown product of THC (Levine 1944; Turk et al 1970) and fresh cannabis has been found to contain little or no CBN (Ohlsson et al 1971; Turner et al 1975; Field & Arndt 1980). Absence of CBN would therefore seem to be a fair criterion of freshness.

Sample extraction

Each sample was analysed as received, except that in the case of herbal material, intact stems were first removed. It was necessary to grind resins as previously described to ensure complete extraction of the cannabinoids (Baker et al 1980c). From a minimum of 5 g of sample, 1 g was extracted with 20 ml of methanol–chloroform (4:1) by ultrasonic vibration for 15 min. A small portion (approx. 2 ml) of the resulting extract was removed and filtered through a cotton wool plug in a Pasteur pipette and analysed by h.p.l.c. and g.l.c. Dilution of this sample with ethanol was sometimes necessary to produce an extract of appropriate strength for analysis.

Gas chromatographic analysis

A Pye 104 gas chromatograph (Pye Unicam, Cambridge, UK) fitted with a flame ionization detector was used. The column was operated isothermally at 250 °C and detector at 300 °C. Injection of 5 μl samples was directly into a 1·5 m × 4 mm i.d. glass column packed with 3% OV-17 on Gas-Chrom Q (100–120 mesh) (Phase-Sep Ltd, Queensferry, UK). Gas flow rates were 30 ml min^{-1} for nitrogen (carrier) and hydrogen, and 450 ml min^{-1} for air. Quantitation was by peak height measurement and comparison with standard solutions of THC.

High performance liquid chromatographic analysis

Analysis of the samples was carried out as previously described (Baker et al 1980c). A constant flow pump (Applied Chromatography Systems, Luton, UK, Model 750/03) was used with ultraviolet detection (Cecil Instruments Ltd, Cambridge, UK, Model 212) at 220 nm. The column was 25 cm × 4 mm i.d. stainless steel packed with octadecylsilane bonded silica (Phase-Sep Ltd, Type S5ODS). 10 mV potentiometric recorders (Bryans Southern Instruments, Mitcham, UK, Model 28000) were used for both g.l.c. and h.p.l.c. The eluting solvent

consisted of a mixture of aqueous 0·01 M sulphuric acid, methanol and acetonitrile (7:8:9). The acetonitrile was h.p.l.c. grade S, maximum absorbance 50% at 205 nm (Rathburn Chemicals Ltd, Walkerburn, UK). The flow rate was 2·5 ml min^{-1}. Aliquots (2 μl) were analysed using on-column stopped-flow injection.

Calculations

THCA (per cent, by weight) was determined by subtracting actual THC, as determined by h.p.l.c., from the 'total' THC, as determined by g.l.c. and multiplying by 1·14 (the ratio of the molecular weights of THCA and THC). Decarboxylation on injection was assumed to be rapid and quantitative as the THC peak was sharp, with no evidence of tailing. No diffuse peaks were observed which could be attributed to residual acids.

RESULTS AND DISCUSSION

Tables 2 and 3 show the mean percentages by weight of THCA, THC and 'total' THC in cannabis and cannabis resin respectively. No significant peak which might correspond to THCA 'B' was detected by h.p.l.c. in any of the samples examined in this study. It was not possible to check whether THCA 'A' and THCA 'B' were separated because no authentic THCA 'B' was available. Whether the two acids are separated or not has had no affect on the data in Tables 2 and 3.

Table 2. THCA, THC and 'total' THC contents of cannabis samples.

	THCA			THC			Total THC			THCA/THC
Country of origin	x	s.d.	c.v.	x	s.d.	c.v.	x	s.d.	c.v.	
Jamaica n = 14	2·3	1·2	52	1·0	0·4	40	3·0	0·8	27	2·3
Kenya n = 5	2·0	0·5	25	1·7	0·5	29	3·4	0·6	18	1·2
South Africa n = 10	2·2	0·9	41	1·2	0·4	33	3·1	0·9	29	1·8
Tanzania n = 2	2·8	—	—	1·5	—	—	4·0	—	—	1·9
Swaziland n = 1	1·6	—	—	2·2	—	—	3·6	—	—	0·7
Nigeria n = 15	3·0	2·2	73	1·9	0·8	42	4·5	1·8	40	1·6
Zimbabwe n = 3	2·3	—	—	1·0	—	—	3·0	—	—	2·3
India n = 4	1·1	—	—	0·4	—	—	1·4	—	—	2·8
India n = 1	8·0	—	—	3·6	—	—	10·6	—	—	2·2
Sri Lanka n = 1	0·3	—	—	0·7	—	—	1·0	—	—	0·4
Thailand n = 4	2·4	—	—	4·9	—	—	6·7	—	—	0·5
USA n = 1	0·4	—	—	5·0	—	—	4·8	—	—	0·1
Columbia n = 1	5·6	—	—	1·9	—	—	6·8	—	—	2·9
Zambia n = 2	1·7	—	—	6·2	—	—	7·7	—	—	3·6

n = number of samples.
x = mean % w/w.
s.d. = standard deviation.
c.v. = coefficient of variation.

Table 3. THCA, THC and 'total' THC contents of cannabis resin samples.

	THCA			THC			Total THC			THCA/THC
Country of origin	x	s.d.	c.v.	x	s.d.	c.v.	x	s.d.	c.v.	
Morocco n = 2	6·7	—	—	1·1	—	—	7·0	—	—	6·1
Turkey n = 1	7·3	—	—	2·3	—	—	8·7	—	—	3·2
The Lebanon n = 7	8·1	4·1	51	1·7	0·8	47	8·8	3·2	36	4·8
Pakistan n = 8	2·7	1·4	52	3·6	1·8	50	6·0	3·0	50	0·8
India n = 8	4·3	3·1	72	8·7	4·5	52	12·5	4·4	35	0·5

Symbols as Table 2.

For forensic purposes, it is often necessary to compare two samples of cannabis or cannabis resin in order to establish (or refute) a connection between them. H.p.l.c. is the principal technique used for comparing the cannabinoid content of samples. For such comparisons to be valid, it is essential to be confident that unrelated samples do not, by coincidence, show similar cannabinoid distribution patterns when subjected to h.p.l.c. In the course of the present and earlier studies carried out in this Laboratory (Baker et al 1980c), no coincidental pattern matching of up to 12 cannabinoids occurred. Similarly, it may be useful to offer an opinion on the origin of a sample. While few conclusions can be drawn from a consideration of the results of the present study in isolation, the use of other h.p.l.c. data and information derived from t.l.c. and macroscopic appearance may help the forensic analyst to offer a firmer opinion.

Most cannabis samples were mature when received, containing flowering and/or fruiting tops. However, four fresh samples from India were immature and low levels of THCA and THC were found. By contrast, a fifth sample was fully mature and contained far higher levels of both of these cannabinoids (Table 2). It may also be that other factors such as environment or plant sex are affecting the cannabinoid levels in these samples.

Cannabis resins from Mediterranean countries (Morocco, The Lebanon, Turkey) are characteristically powdery and are pale green/brown, while those from India and Pakistan are much darker, varying from brown to almost black (de Faubert Maunder 1976). Furthermore, in marked contrast to Mediterranean resins where a high ($\gg 1$) THCA to THC ratio is found, Pakistan and Indian resins have much lower values (generally <1).

Although there are small numbers of samples of cannabis or cannabis resin from some countries, it is apparent from Tables 2 & 3 that wide variations in the relative amounts of THCA and THC occur and to a slightly lesser extent in total THC even in samples of the same geographical origin. Although variations in the relative amounts of THCA and THC do not affect the potency of the smoked material, considerable variations in effect may occur when the material is ingested as THCA is inactive orally. Cannabis preparations are widely taken orally in India (Chopra & Chopra 1957) and it may be that the demand for a material active for eating is reflected in the method of preparation of Indian cannabis resin.

Razdan (1972) considered that some of the discrepancies in the published biological data on cannabis products and their effects on physiological systems were due to the presence of both acidic and neutral cannabinoids in the samples under examination. The results that we have described in this paper demonstrate that in order to investigate fully the pharmacological and chemobotanic properties of cannabis products, it is essential that the chemical constitution and method of administration of the material under study is fully described.

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