J. Pharm. Pharmac., 1977, 29, 491–494 *Received January* 14, 1977

Cannabinoid patterns in seedlings of *Cannabis sativa* L. and their use in the determination of chemical race

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We have examined the cannabinoid contents of seedlings from twelve strains of *Cannabis* of known chemical race. Fourteen days after emergence of the shoot those of the tetrahydrocannabinol (THC) type could be distinguished from those of the cannabidiol (CBD) type. The true leaves of the THC type contained a relatively high content of THC and also contained cannabichromene (CBC), sometimes as the major cannabinoid, whereas the CBD type had much lower amounts of THC and no CBC; CBD being the major component. A strain from China corresponded to the THC type but showed some unusual features. Seeds purchased at seven outlets in Britain were also examined. Only two batches germinated but these proved to be of a THC type and resembled the Chinese strain.

It is well known that *Cannabis sativa* L. shows a number of chemical races. Fettermann, Keith & others (1971) suggested two, one containing predominantly Δ^1-tetrahydrocannabinol (THC) and one mainly cannabidiol (CBD) corresponding to 'drug type' and 'fibre type' respectively. Fairbairn & Liebmann (1974) grew 12 strains in England and found that most were either THC-rich or CBD-rich; there was some evidence of an intermediate race with roughly equal concentrations of THC and CBD. The difference between the two major types was maintained when several of them were grown in Canada, U.S.A., Norway, Turkey and Thailand (Fairbairn, 1976).

More recently, Small, Beckstead & Chan (1975) grew a much larger number of strains and proposed four chemical races. Two correspond to the THC-rich and CBD-rich races, a third is intermediate, and the fourth contains THC predominantly with trace amounts of cannabigerol monomethyl ether. This last race occurs in N.E. Asia. Small, Jui & Lefkovitch (1976) later claimed to have confirmed by numerical taxonomic analysis of 2500 plants that four comprehensive groups can be recognized in the single species *C. sativa*.

Since cannabis seeds are sold without legal restrictions and yet plants can be readily raised from them in most climates, we decided to investigate whether one could predict the chemical race by examination of the cannabinoid pattern in young seedlings. The seeds themselves contain no cannabinoids and although some authors claim that morphological characters can be used to indicate the type of plant which will be produced, Small (1975 with references) has shown this is not so. The characters used—seed size, presence or absence of a complete perianth and elongated base—merely distinguish seeds of wild plants from those of cultivated ones. Intoxicant and non-intoxicant types fall into both categories.

Little work has been done on the appearance of cannabinoids after germination. Maunder (1970) using t.l.c. methods, found detectable amounts of THC in all seedlings examined even where the cotyledons were not fully developed. Krejci (1970) demonstrated antibiotic activity from the eighth week and this was probably due to the presence of CBD. Turner, Fetterman & others (1975a) monitored the cannabinoid pattern of a Mexican strain from the fifth week onwards. However, for control methods and other legal problems, a knowledge of the detailed patterns shortly after germination would be more useful.

We have already published a preliminary note on our work (Fairbairn & Rowan, 1975) and since then Rasmussen & Herweijer (1975) have reported work on seedlings. Their results will be discussed later.

MATERIALS AND METHODS

The cannabis seeds used and their geographical origin are shown on Table 1. Those labelled 'UN etc.' were supplied by the United Nations Division of Narcotics, Geneva. (MA9C and ME-A3 were given to us by Dr C. E. Turner and SP-14 was supplied by Messrs C. A. Gould from a consignment recently imported from China). Most of these strains had

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Table 1. *Cannabinoid content in mg g^{-1} dry weight of the true leaves of 14 day old seedlings of known type and geographical origin.*

Strain	Origin	No analysed	THC cont. mean (+ range)	CBD + CBC cont. mean (+ range)
Known THC types				
ME-A3	Mexico	10	6·4 (1·8–10·6)	4·4 (2·3–10·9)
UNC 254	Thailand	9	2·2 (1·1–2·9)	2·2 (1·3–3·0)
UNS-1	South Africa	9	2·5 (0·9–6·3)	0·9 (0·4–1·3)
SP-2	Nepal	10	2·3 (1·1–3·9)	2·6 (1·3–4·4)
SP-8	India	7	0·8 (0·4–1·4)	0·8 (0·4–1·8)
UNC335-E	South Africa	10	2·3 (1·3–4·3)	1·3 (0·7–2·3)
UNC347	Mexico	10	1·1 (0·3–2·8)	1·1 (0·3–2·2)
UNC340	Nepal	10	1·7 (0·6–2·4)	1·5 (0·7–2·3)
SP-14	China	10	0·4 (0·1–1·2)	9·4[a] (5·2–17·8)
Known CBD types				
UNC354	Turkey	10	<0·02 (0–t)	7·1[b] (5·1–10·6)
SP-1-E	Turkey	9	<0·02 (0–t)	5·2[b] (3·8–7·8)
SP-13	Kew	10	0·02 (0–0·06)	1·6[b] (0·9–2·7)

[a] Examination by t.l.c. showed that this was mainly CBC.
[b] No CBC was observed in these seedlings when the extracts were examined by t.l.c.
t. Trace.

been grown in our botanical garden in previous years so that the cannabinoid pattern of the mature plants was known (Fairbairn & Liebmann, 1974). Further samples of cannabis seed were bought at retail outlets in Welwyn Garden City, Hatfield, Thetford, Rotherham, Cardiff, Glasgow and Sunderland. All samples were examined for the morphological characters described by Small (1975).

A THC-rich strain (MA9C) and a CBD-rich strain (UNC 354) were sown in a greenhouse and ten seedlings of each were harvested as soon as they emerged and then daily (in mid-afternoon) for 28 days. Each batch of seedlings was bulked to overcome individual variation and sampling error, and cotyledons, stem and true leaves were analysed separately.

The other strains were also grown in a greenhouse and harvested 14 days after emergence of the shoots and the true leaves of each of the seedlings were analysed separately. One leaf only was taken from each seedling of the Rotherham and Sunderland strains, the seedlings being subsequently grown to maturity.

Quantitative analysis was carried out using a scaled down modification of the method of Fairbairn & Liebmann (1973). This method does not separate cannabichromene (CBC) from CBD and so qualitative identification of these cannbinoids was checked by gas liquid chromatography (g.l.c.) on a column of OV-1 (6%) (Turner, Hadley & others, 1975b) and by thin layer chromatography (Maunder, 1969). Some samples were also examined by combined gas chromatography-mass spectroscopy (g.c.-ms) (carried out at the Physiochemical Measurements Unit, Harwell).

RESULTS

The appearance of cannabinoids in the leaves during early stages of development of a THC-rich and a CBD-rich strain are shown in Fig. 1. During the first few days many unidentified peaks were observed by g.l.c. Some of these peaks overlapped those of cannabinoids and made accurate assay of the latter difficult. By 14 days the chromatograms were much simpler and a clear difference between the THC-rich and CBD-rich strains could be observed.

The seedling stems showed a pattern similar to that in Fig. 1 but they contained less total cannabinoid (about 30% of that in the leaves). G.c.-ms

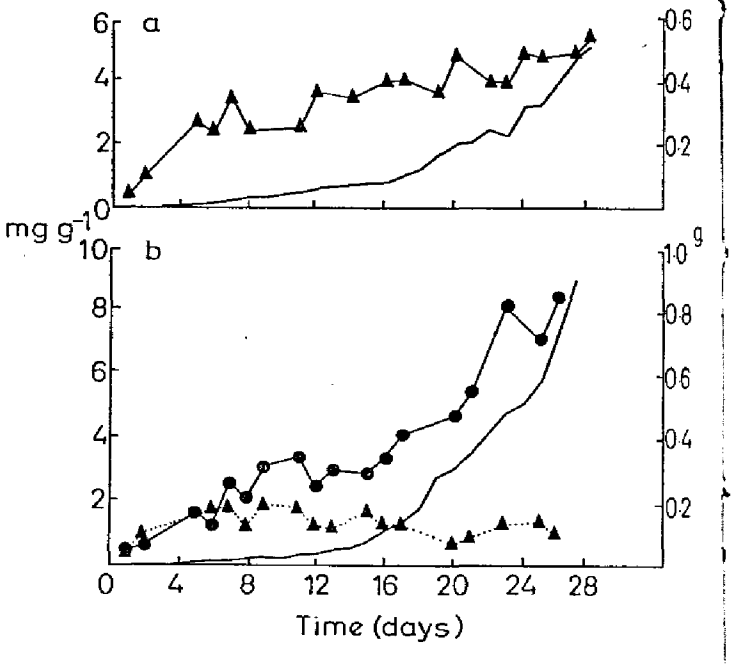

FIG. 1. Cannabinoid content (mg g^{-1}) of the true leaves of seedlings of a—UNC 354 and b—MA9C during the first month of growth. THC content (●—●), CBD + CBC content (▲ - - - ▲); and CBD content ▲—▲. Solid line indicates dry weight per seedling (g).

examination showed that two of the prominent peaks were the hydrocarbons nonacosane and heptacosane. These had relative retention times of 0·67 and 0·31 respectively on the OV-17 g.l.c. system and tended to overlap with the peaks of cannabinol and CBD

(0·60 and 0·35 respectively). Turner & Hadley (1973) have pointed out that nonacosane may be mistaken for a cannabinoid if only g.l.c. data is used, and clearly heptacosane could similarly be misinterpreted. The cotyledons also contained these hydrocarbons but at no stage did they contain cannabinoids.

It was decided to use the leaves of 14-day old seedlings for a survey of the cannabinoid content of the remaining strains and the results are given in Table 1. Qualitative analysis by t.l.c. showed that the single CBC/CBD peak of THC types was predominantly CBC, whereas the CBD-rich types had no detectable CBC. In all the seedlings the cannabinoids were mainly present as their acids.

The recently purchased batches of seed (of unknown race) were next examined. Germination rates were poor: Sunderland (8%) and Rotherham (5%). The remaining batches did not germinate at all. The results of quantitative analysis of seedlings of 14 days and of mature plants are given in Table 2.

Table 2. *Cannabinoid content in mg g^{-1} dry weight of the true leaves of 14 day old seedlings and mature plants grown from seeds bought at British, retail outlets.*

	Cannabinoid content of seedling		Cannabinoid content when mature[a]	
Plant	THC	CBD and CBC[b]	THC	CBD and CBC
Rotherham				
1	0·3	8·0	7·5	t
2	1·3	9·6	3·6	2·5
3	0·7	17·0	4·1	2·7
4	0·7	10·1	3·2	t
5	0·8	10·7	plant died	
Mean	0·76	11·08	4·6	1·3
Sunderland				
1	0·5	4·9	13·8	4·0
2	0·08	3·3	7·0	t
3	0·2	2·9	2·1	t
4	0·3	4·1	4·0	t
5	0	9·8	3·7	t
6	0·4	4·4	8·0	t
7	0·2	1·8	11·3	t
Mean	0·24	4·46	7·13	0·6
SP-14 Mean	0·4	9·4	8·8	t

[a] When mature the Rotherham and Sunderland plants also contained a compound with a relative retention time similar to that of tetrahydrocannabivarin and cannabicyclol.

[b] Examination by t.l.c. showed that this was mainly CBC.

t. Trace.

T.l.c. examination showed that CBC was the predominant cannabinoid in the seedlings.

DISCUSSION

At no stage do the cotyledons contain cannabinoids although hydrocarbons such as nonacosane and heptacosane occur and could be confused with cannabinoids in normal gas chromatography. At the appearance of the first true leaves, however, cannabinoids begin to appear in the leaf and to a lesser extent in the stem. At this stage the seedling can be considered to have an existence independent of the seed. Our value for the Mexican strain at 4 weeks (0·8% THC) agrees well with that of Turner & others (1975a) at 5 weeks (0·5% THC), especially in view of the fact our plants were raised under different conditions.

The co-existence of hydrocarbons and other unidentified peaks in the t.l.c. traces during the early stages makes quantitative analysis difficult. At 14 days the picture is much simpler and we recommend that the analysis be carried out then. At this stage it is possible to determine the chemical race as follows:

(a) The THC-rich race has a much higher absolute amount of THC than the CBD-rich race. The first 8 samples (Table 1), had values ranging from 0·8 to 6·4 mg THC g^{-1}, whereas in the CBD types the values were less than 0·02 mg g^{-1}.

(b) The THC-rich race always possesses cannabichromene (CBC) sometimes in large amounts. None of the CBD-rich samples we examined possessed CBC. This difference can be detected by t.l.c. Rasmussen & Herweijer (1975) also report the absence of cannabinoids from the cotyledons at all stages of development. They worked mainly on the Thailand strain (UNC 254) and claim that CBD is the predominant cannabinoid in the seedlings. This conclusion is based on g.l.c. results only: our work on the same strain shows there is a prominent peak at the position of CBD but t.l.c. investigation showed it to be partly due to cannabichromene (CBC) which agrees with the fact that UNC. 254 is a THC-rich strain.

The sample from China (SP-14, Tables 1 and 2) falls into the THC-rich category as the seedlings produce a large amount of CBC, and the amount of THC present in significantly greater than in the CBD-rich race. However, the amount of THC is much smaller than in the remaining 8 samples of the THC-rich group and this applies to the mature plant. The Chinese sample had 8·8 mg THC^{-1} g in mature

female tops: for the other 8 samples the corresponding values range from 18 to 71 mg THC g^{-1} (Fairbairn & Liebmann, 1974). Our Chinese sample may therefore represent the fourth chemical race proposed by Small & others (1975) and which occurs in N.E. Asia, and it is interesting that Shoyama, Fujita & others (1968) reported on a Japanese strain which also contained CBC as the major cannabinoid in young plants.

Small & others (1975) state that one distinguishing feature of this race is the presence of cannabigerol monomethylether. We examined SP-14 material and did find a very small peak on the g.l.c. with a retention time identical to pure cannabigerol monomethylether, but it was too small to separate.

It would seem from Table 2 that the Rotherham and Sunderland seeds were also of this fourth type; this is not surprising as 95% of the cannabis seeds imported into the U.K. at present are from China. Although the viability of samples purchased legally is poor, those that do germinate will produce reasonably potent cannabis when grown in this country.

Morphological examination of the seeds confirmed Small's conclusion (1975) that these characters cannot be used to distinguish THC-rich from CBD-rich races. Almost all of the samples in Table 1 showed the marbling effect due to the reticulate venation of the mesocarp; traces of the hyaline perianth were occasionally present and the base was usually flat or very slightly elongated: the sizes ranged from about 4 to 5 mm. Sample UNC 340 (Nepal) was markedly smaller (2½ to 3 mm) and did possess more perianth and had, in some seeds, a slightly elongated base. These results may indicate that all our seed samples are from cultivated plants except UNC 340 which may have been from the wild.

Acknowledgements

We would like to thank Professor F. Fish, Dr B. P. Jackson and Mr T. D. Turner for samples of seeds.

REFERENCES

FAIRBAIRN, J. W. & LIEBMANN, J. A. (1973). *J. Pharm. Pharmac.*, **25**, 150–155.

FAIRBAIRN, J. W. & LIEBMANN, J. A. (1974). *Ibid.*, **26**, 413–419.

FAIRBAIRN, J. W. & ROWAN, M. G. (1975). *Ibid.*, **27**, *Suppl.*, 90P.

FAIRBAIRN, J. W. (1976). The Pharmacognosy of Cannabis, In: *Cannabis and Health*, pp. 3–19. Editor: Graham, J. D. P. London: Academic Press.

FETTERMAN, P. S., KEITH, E. S., WALLER, C. W., GURRERO, O., DOORENBOS, N. J. & QUIMBY, M. W. (1971). *J. pharm. Sci.*, **60**, 1246–1249.

KREJCI, Z. (1970). In: *The Botany and Chemistry of Cannabis*, pp. 49–55. Editors: Joyce, C. R. B. and Curry, S. H., London: J. and A. Churchill.

MAUNDER, M. J. DE FAUBERT (1969). *J. Pharm. Pharmac.*, **21**, 334–335.

MAUNDER, M. J. DE FAUBERT (1970). *J. Ass. Publ. Analysts*, **8**, 42–47.

RASMUSSEN, K. E. & HERWEIJER, J. J. (1975). *Pharm. Weekblad*, **110**, 91–93.

SHOYAMA, V., FUJITA, T., YAMAUCHI, T. & NISHIOKA, I. (1968). *Chem. Pharm. Bull.*, **16**, **1157**.

SMALL, E., BECKSTEAD, H. D. & CHAN, A. (1975). *Econ. Bot.*, **29**, 219–232.

SMALL, E. (1975). *Can. J. Bot.*, **53**, 978–987.

SMALL, E., JUI, P. Y. & LEFKOVITCH, L. P. (1976). *Syst. Bot.*, **1**, 67–84.

TURNER, C. E. & HADLEY, K. W. (1973). *J. pharm. Sci.*, **62**, 1083–1086.

TURNER, C. E., FETTERMAN, P. S., HADLEY, K. W. & URBANEK, J. E., (1975a). *Acta Pharm. Jugosl.*, **25**, 7–16.

TURNER, C. E., HADLEY, K. W., HENRY, J., BILLETS, S. & MOLE, M. L. (1975b). *J. pharm. Sci.*, **64**, 810–814.