J. Pharm. Pharmac., 1974, **26**, 413–419 *Received November* 14, 1973

The cannabinoid content of *Cannabis sativa* L grown in England

J. W. FAIRBAIRN AND J. A. LIEBMANN

Department of Pharmacognosy, The School of Pharmacy, University of London, Brunswick Square, London WC1 1AX, U.K.

Twelve varieties of *Cannabis sativa* were grown out-of-doors in southern England during 1971 to 1973. Results show that for certain varieties highly active herbal cannabis can be produced. A warm climate with abundant sunshine does not therefore seem to be essential for high THC content. This was supported by results of growing plants in a greenhouse in varying lighting conditions including a limited period in total darkness. Considerable within and between plant variation was found and the importance of defining the plant part used, the stage of growth and the size of the sample is emphasized for comparative work involving quantitative results. Comparison of the present results with those for the same cannabis varieties grown in different parts of the world shows that all exhibit the same qualitative picture, that is, either THC-rich or CBD-rich. Since this chemical composition seems independent of environmental conditions it is inappropriate to refer to the two types as phenotypes; it is more likely that they represent two chemical races within the species *Cannabis sativa* L.

There has recently been wide interest in the production of cannabinoids in *Cannabis sativa* L. The concentration within the plant of the major psychoactive constituent, $\Delta^1(\Delta^9)$-tetrahydrocannabinol (THC), is known to vary widely (De Faubert-Maunder, 1970; Ohlsson, Abu-Charr & others, 1971; Waller, 1971) and much of the interest has centred on the factors that influence the production of THC. Those considered have included the genetic background, the geographical origin and the sex of the plant (Davis, Farmilo & Osadchuk, 1963; Valle, Lapa & Barros, 1968; Ohlsson & others, 1971; Waller, 1971; Fetterman, Keith & others, 1971). Attention has also been given to the distribution of THC in various parts of the plant (Fetterman & others, 1971) and the changes in THC content during its growth (Phillips, Turk & others, 1970). It has been suggested (Davis & others, 1963) that plants grown in temperate climates produce much less THC than those from hotter regions, although more recent results indicate that the effect of climate itself may not be marked (De Faubert-Maunder, 1970; Ohlsson & others, 1971; Waller, 1971). Some estimates of the THC content of plants grown in the cool climate of north-west Europe have appeared (De Faubert-Maunder, 1970; Nielsen, 1970; Fairbairn, Liebmann & Simic, 1971b; Ohlsson & others, 1971) but these are limited in value since the analytical methods used do not give an accurate indication of the total available THC. Work on plants grown in southern U.S.A. based on reliable g.l.c. analysis indicates that quite active material can be produced there (Doorenbos, Fetterman & others, 1971). Small & Beckstead (1973) claim that seed from countries north of 30° latitude produced plants low in THC when grown in Ottawa, Canada.

We now report on the cannabinoid content of twelve varieties grown out-of-doors

in southern England and on the variation in content both within and between plants. The effects of growing plants under varying conditions in the greenhouse, have also been investigated.

MATERIALS AND METHODS

Material

Varieties labelled "UN etc." in the Tables were grown from seeds supplied by the U.N. Division of Narcotics, Geneva; the remainder were grown from seeds obtained as follows: SP1—purchased in Merzifon, Turkey, 1969. SP2—purchased in Katmandu, Nepal, 1970. SP5—collected from plants seized in N. London by the police, 1971. SP8—from India, 1971, labelled "Cannabis (ganja) seeds".

Cultivation

To ensure a supply of plants each season, seeds were sown in pots in March in the greenhouse (10–15°) and transplanted out-of-doors at the end of May. Seeds were also sown directly out-of-doors at the end of May and the plants soon reached the height and vigour of the corresponding varieties from greenhouse-started plants. Hence the ages in Table 1 refer to time from transplanting or sowing out-of-doors. Normal horticultural procedures were used and samples were collected from August to November, as indicated in the Tables. The following figures of average monthly rainfall, sunshine and temperatures from the records of a local meteorological station, give some idea of the weather conditions prevailing during these months.

		Rainfall (mm)	Temperatures (°C) Min	Max	Sunshine (h)
1971:	June	106·9	6·7–12·9	12·3–23·3	146
	July	23·9	6·7–17·1	18·3–28·9	207
	Aug	90·6	9·4–16·1	18·3–26·7	146
	Sept	18·1	5·6–13·6	14·5–25·0	178
	Oct	62·9	1·8–13·9	10·0–22·8	148
	Nov	67·7	(—2·3)–8·3	4·4–16·6	100
1972:	June	38·2	5·9–13·0	13·5–20·0	188
	July	59·9	8·0–16·0	16·8–26·7	165
	Aug	24·9	7·8–16·4	17·3–25·0	188
	Sept	35·8	4·3–12·9	11·6–22·6	133
	Oct	12·2	3·2–12·9	10·5–22·0	117
	Nov	72·3	1·0–13·0	3·8–16·0	84

Collection of samples

Samples were collected according to the following arbitrary definitions: (a) *Leaves*, healthy leaves from plants at vegetative or flowering stages and which were not closely associated with a floral axis. (b) *Vegetative tops*, the crowded mass of small leaves at the ends of the shoot. (c) *Flowering tops*, the crowded mass of bracts, flowers and immature fruits at the ends of the flowering shoots. Wherever possible they were collected when the stigmas of the oldest flowers were beginning to wilt. Stalks or stems above 2 mm diameter were removed from all samples which were then dried in the shade in a current of air for 3 days and stored in a cool dark place.

Analysis

The cannabinoids were determined by the g.l.c. method of Fairbairn & Liebmann (1973). During our work Turner & Hadley (1973) published evidence to show that the g.l.c. peak normally assumed to be cannabidiol (CBD) may, in some varieties, be due to cannabichromene or cannabivarin. Each variety analysed was therefore

Table 1. *Cannabinoid content (% of air-dried material) of varieties grown out-of-doors.*

	Variety	Growth stage and part collected	Age[a] and date collected	Cannabinoids THC	CBN	CBD
1.	S. African	Veg. tops	19 wk Oct 71	1·86	—	—
	UN S1	Fl.[c] tops	19 wk Oct 71	2·13	—	—
2.	S. African	Veg. 1 m[d] leaf	11 wk July 72	0·46	—	*b*
	UNC 255	Veg. 1 m leaf	5 wk June 73	1·29	—	*b*
		Veg. 2 m leaf	15 wk Aug 72	2·34	—	*b*
		Fl. tops (1st plant)	25 wk Oct 72	4·60	—	*b*
		Fl. leaf (1st „)	25 wk Oct 72	2·27	—	*b*
		Fl. tops (2nd „)	25 wk Oct 72	3·37	—	*b*
		Fl. leaf (2nd „)	25 wk Oct 72	1·90	—	*b*
3.	S. African	Veg. 1 m leaf *	12 wk July 72	1·62	—	*b*
	UNC 335	Veg. 2 m tops *	15 wk Aug 72	6·88	—	*b*
		leaf	15 wk Aug 72	0·75	—	*b*
		Fl. tops (1st plant)	22 wk Oct 72	7·12	—	*b*
		Fl. leaf (1st „)	22 wk Oct 72	4·50	—	*b*
		Fl. tops (2nd „)	22 wk Oct 72	3·70	—	*b*
		Fl. leaf (2nd „)	22 wk Oct 72	3·00	—	*b*
		Fl. tops (3rd „)	27 wk Nov 72	3·37	—	*b*
4.	Thailand	Veg. leaf	14 wk Sep 71	1·16	—	*b*
	UNC 254	Veg. tops	18 wk Oct 71	1·45	0·15	*b*
		Veg 1 m leaf	10 wk July 72	0·28	—	*b*
		Veg. 2 m leaf *	14 wk Aug 72	0·87	—	*b*
		Veg. 2 m tops *	14 wk Aug 72	2·41	—	*b*
		Veg. 1 m leaf	5 wk June 73	0·74	0·09	*b*
		Fl. tops	18 wk Oct 71	1·80	0·18	*b*
		Fl. tops	24 wk Oct 72	2·43	—	*b*
		Fl. leaf	24 wk Oct 72	0·92	—	*b*
5.	Indian	Veg. 2 m tops *	14 wk Aug 72	3·62	—	*b*
	SP 8	Veg. 2 m leaf *	14 wk Aug 72	1·16	—	*b*
		Veg. 3 m tops *	22 wk Oct 72	4·03	—	*b*
		Veg. 3 m leaf *	22 wk Oct 72	2·29	—	*b*
6.	Nepalese	Veg. tops *	21 wk Oct 71	1·33	—	*b*
	SP 2	Veg. leaf *	21 wk Oct 71	0·14	—	*b*
		Fl. tops	21 wk Oct 71	2·70	—	*b*
7.	Nepalese	Veg. 1 m leaf	20 wk Sep 72	0·86	—	—
	UNC 340	Veg. 1 m leaf	5 wk June 73	0·66	—	*b*
8.	Leyton	Veg. 0·8 m tops	15 wk Aug 72	5·32	—	*b*
	SP 5	Veg. 0·8 m leaf	15 wk Aug 72	2·54	—	*b*
		Fl. tops	24 wk Oct 72	4·85	—	—
9.	Mexican	Veg. 1 m	18 wk Sep 72	1·21	—	*b*
	UNC 347	Veg. 1 m	5 wk June 73	1·51	—	—
10.	Turkish	Veg. 3 m tops	26 wk Nov 72	0·04	—	1·10
	SP 1	Veg. 3 m leaf	26 wk Nov 72	0·02	—	0·20
		Fl. tops	26 wk Nov 72	0·10	—	1·69
		Fl. leaf	26 wk Nov 72	0·33	—	0·88
11.	Turkish	Veg. 1 m	11 wk July 72	0·01	—	0·12
	UNC 258	Fl. tops	22 wk Oct 72	0·07	—	0·94
		Fl. leaf	22 wk Oct 72	0·02	—	0·21
		Veg. 1 m leaf	5 wk June 73	—	—	0·66
12.	French	Veg. 2 m tops	15 wk Aug 72	0·06	—	1·10
	SP 9	Veg. 2 m leaf	15 wk Aug 72	0·05	—	0·27

Notes:

a = age in weeks from transplanting or sowing out-of-doors.

b In these varieties a peak corresponding to CBD occurred but t.l.c examination indicated CBD was absent.

c Fl. = flowering stage, female shoots.

d Veg. = vegetative stage: height in metres (m).

* See discussion p. 418

checked for the presence of CBD by t.l.c. (De Faubert-Maunder, 1969); if shown to be absent the "CBD" peak in g.l.c. was ignored.

RESULTS

Table 1 gives the results for twelve varieties grown out-of-doors during 1971 and 1972 (and a few for 1973). Each result was based on one plant and as there seemed to be wide variation in cannabinoid content within some varieties, the between-plant variation was examined. Pairs of plants of the same variety grown in similar conditions were analysed separately and the results are shown in Table 2. Later, 16

Table 2. *Variation in cannabinoid content (% of air-dried material) between plants of the same variety grown in identical conditions out-of-doors (unless otherwise stated) and collected simultaneously.* Veg. = vegetative phase, height in metres (m)

Variety	Details of plant		Cannabinoid content THC	CBN	"CBD"*
South African	Veg. 1 m leaf 1st plant		0·67	—	0·03
UNC 255	,, 2nd plant		0·20	—	0·01
UNC 335	Veg. 1 m leaf 1st plant		2·38	—	0·11
	,, 2nd plant		0·89	—	0·03
Thailand	Veg. 1 m leaf 1st plant		0·27	—	0·03
UNC 254	,, 2nd plant		0·29	—	0·04
	Veg. 0·8 m leaf (greenhouse)				
	(a) *Normal lighting*				
	8 plants	Means	0·91	0·11	0·11
		c.v.	21%	13%	66%
	(b) *After 21 days in dark*				
	8 plants	Means	0·81	0·13	0·13
		c.v.	28%	11%	52%

* Although no CBD was present, the quantities of cannabinoid present (cannabichromene and cannabivarin, Turner & Hadley, 1973) were calculated as CBD, to illustrate variation in these components.

greenhouse-grown plants of variety UNC 254 (vegetative phase, height 80 cm) were divided into two equal groups. One group was allowed to continue growing under normal greenhouse conditions and the other in complete darkness for 21 days. The leaves from each plant were collected and dried: from the plants grown in the light the mean weight of leaf per plant was 4·9 g (s.d. 1·79) and for those grown in the dark 2·3 g (s.d. 0·83). The cannabinoid content for each plant was also determined, the means and standard deviations calculated (Table 2).

Greenhouse experiments were also carried out to determine whether increased light intensity or additional ultraviolet light would lead to increased production of cannabinoids. Four similar groups of the Nepalese variety (SP2), three plants each, were grown in the following conditions: (a) normal greenhouse daylight, (b) normal lighting supplemented by 3 h exposure to intense illumination from five 400 W Philips daylight lamps at the beginning of the day and 3 h at the end, (c) normal lighting supplemented by additional ultraviolet irradiation from two 400 W Osram UV lamps for 2 h at the beginning and 2 h at the end of the day, (d) out-of-doors. The results of analysis for THC content of the upper leaves (% air dried material) were for (a) 2·4, (b) 2·36, (c) 2·96 and (d) 4·42.

As the results in Table 1 also indicated within-plant variation, equivalent samples of leaves were collected at different levels from young plants (vegetative phase) and the results are shown in Table 3.

Table 3. *THC content (% of air-dried material) and air-dry weight of leaves collected simultaneously at different positions on the plant.*

Position on plant		Plant 1 (SP5)	Plant 2 (SP5)	Plant 3 (UNC 335)
Top	THC	6·1	6·9	4·8
	Dry weight	35 mg	21 mg	28 mg
Middle	THC	3·0	5·5	3·1
	Dry weight	119 mg	74 mg	70 mg
Bottom	THC	0·8	4·0	1·5
	Dry weight	314 mg	133 mg	133 mg

DISCUSSION

Highly active plants grown in temperate climate

The results in Table 1 show that the dried flowering or vegetative tops (herbal cannabis) of some of the varieties are surprisingly active. For the South African varieties the figures vary from 1·86 to 7·12% THC; the Thailand variety 1·45 to 2·43% and the "Leyton" SP5, 4·85 to 5·32% THC. These figures compare favourably with those for the more concentrated cannabis resin usually imported from semi-tropical regions (for example, median values of 4·82, 1·30 and 5·40% THC for three groups of seized resin. Cannabis Report, 1972). The meteorological data given earlier indicate that our plants were grown in temperate conditions and this, therefore, disproves that a hot sunny climate is necessary for producing active cannabis.

Effect of light intensity

The work on growing plants in differing light intensities (see above) indicates a similar conclusion as samples (b) and (c) which had supplementary lighting had no higher cannabinoid content than the two "controls" (a) and (d). More striking confirmation comes from the fact that no significant increase in the concentration of cannabinoids occurred after exposing healthy plants for 21 days to normal lighting conditions compared with a control group kept in total darkness (Table 2). However the mean dry weight of the leaves of the control plants was only 2·3 g; that of the light plants had increased to 4·9 g. The *absolute* amounts of cannabinoid therefore were 24·8 mg and 55·4 mg respectively. That is, 21 days additional photosynthetic activity had led to an increase in dry weight of 113% and of cannabinoid of 127%. At least in these early stages of growth therefore the cannabinoids do not appear to be metabolized to non-cannabinoid substances, but accumulate with the general increase in dry weight.

A cool climate and poor lighting conditions do not, therefore, seem to prevent the production of active material and we have recently analysed three illicit samples grown in England and have obtained values of 1·98, 2·18 and 2·39% mg THC. Each sample had been collected before flowering and therefore was not, by the present legal definition, cannabis (Fairbairn, Liebmann & Simic, 1971a). It is not possible to say

that if these active varieties had been grown in sunnier and warmer climates they would not have an even higher THC content, without world-wide collaborative work.

Between- and within-plant variation

Table 2 illustrates the marked variation in cannabinoid content between plants from the same variety grown as far as possible in identical conditions. Even within one plant there is great variation; from the results in Table 1 (samples 3 to 6 pairs marked *) it can be seen that the immature leaves of the vegetative tops contain 8·0, 2·8, 3·1, 1·8 and 9·5 times respectively more THC than the lower leaves on the same shoot. A similar positional variation occurs with normal leaves (as distinct from "tops"); the leaves from three greenhouse grown plants at the vegetative phase showed decreases in percentage THC from upper, middle to lower positions of 8:4:1; 1·7:1·4:1; and 3:2:1 respectively (Table 3). This may explain why some of our values are higher than other published figures; e.g. a South African variety (UNC 255) for which Turner & Hadley (1973) give values of 1·18 and 1·6% THC for manicured flowering tops (large stems and fruits removed by suitable sifting). For the same variety Small & Beckstead (1973) give 1·07 and for the Thailand variety UNC 254, 0·66% THC respectively at the vegetative stage (15 weeks old). Doorenbos & others (1971) reported values ranging from 1·7 to 7·2% THC and 1·5 to 4·8% THC for manicured marihuana from male and female plants respectively for a Mexican strain, although no details of sampling methods are given. For collaborative work in different parts of the world, it is therefore essential to take account of the within and between plant variation by defining the parts used strictly and collecting from an adequate number of plants. The stage of development is important and "physiological age" is probably better than chronological age from the time of sowing of the seeds. Thus samples at the vegetative stage would be from plants at a particular height, and flowering samples from fertile *shoots* whose flowers are at a defined stage of fertilization.

Are there two chemical races?

Some, or all, of the varieties 1 to 4, 9 to 12 (Table 1) have now been grown in Canada (Small & Beckstead 1973), Mississippi (Fetterman & others, 1971), Norway (Nordal & Braenden, 1973), Holland and Turkey (private communications) as well as in the U.K. In every instance they have exhibited the same *qualitative* picture; either THC- or CBD-rich. In such circumstances it seems inappropriate to call these types "Phenotype I and II" (Fetterman & others, 1971) or "cannabinoid phenotypes" (Small & Beckstead 1973). Phenotypes vary in response to changing environmental conditions; they are the varying outward expressions of a fixed factor, the genotype (Davis & Heywood, 1963). The morphological features are extremely variable (Quimby, Doorenbos & others, 1973) but results todate indicate that THC and CBD predominance are independent of environmental conditions. Obviously, this constancy must be tested more widely and especially through several generations, but if confirmed, we would be justified in speaking of two chemical races. There is some evidence of the existence of an intermediate type with approximately equal proportions of THC and CBD. Examples of this are the unusual looking bushy plant from Nepal which had ratios of THC to CBD of 1·6 to 2 (Fairbairn & others, 1971b) and plants reported by Small & Beckstead (1973) with a mean ratio of 0·72 for the female plants.

Acknowledgments

We would like to thank the Medical Research Council for a grant towards the cost of this work, Dr. S. Simic and Dr. M. G. Rowan for some analytical assistance and Dr. O. Braenden, Sir Harry Greenfield, Dr. P. N. Suwal, Professor C. Salemink and Professor C. Tariman for certain samples of seeds.

REFERENCES

"CANNABIS. A report of the Commission of Inquiry into the non-medical use of drugs" (1972), p. 28 Information Canada, Ottawa.

DAVIS, P. H. & HEYWOOD, V. H. (1963). *Principles of Angiosperm Taxonomy*, p. 336. London: Oliver and Boyd.

DAVIS, T. W. M., FARMILO, C. G. & OSADCHUK, M. (1963). *Analyt. Chem.*, **35**, 751–755.

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

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

DOORENBOS, N. J., FETTERMAN, P. S., QUIMBY, M. W. & TURNER, C. E. (1971). *Ann. N.Y. Acad. Sci.*, **191**, 3–12.

FAIRBAIRN, J. W., LIEBMANN, J. A. & SIMIC, S. (1971a). *J. Pharm. Pharmac.*, **23**, 558–559.

FAIRBAIRN, J. W., LIEBMANN, J. A. & SIMIC, S. (1971b). *Acta pharmaceutica suecica*, **8**, 679.

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

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

NIELSEN, E. (1970). *Dansk Tidsskr. Farm.*, **44**, 359–364.

NORDAL, A. & BRAENDEN, O. (1973). *Norsk Farmasoytisk. Selskap*, **35**, 8–15.

OHLSSON, A., ABU-CHAAR, C. I., AGURELL, S., NILSSON, I. M., OLOFSSON, K. & SANDBERG, F. (1971). *Bull. Narc.*, **23** (1), 29–32.

PHILLIPS, R., TURK, R., MANNO, J., JAIN, N. & FORNEY, R. (1970). *J. forensic Sci.*, **15**, 191.

QUIMBY, M. W., DOORENBOS, N. J., TURNER, C. E. & MASOUD, A. (1973). *Economic Botany* **27** (1), 117–127.

SMALL, E. & BECKSTEAD, H. D. (1973). *Lloydia*, **36**, 144–165.

TURNER, C. E. & HADLEY, K. (1973). *J. pharm. Sci.*, **62**, 251–255.

VALLE, J. R., LAPA, A. J. & BARROS, G. C. (1968). *J. Pharm. Pharmac.*, **20**, 798–799

WALLER, C. W. (1971). *Proc. West. Pharm. Soc.*, **14**, 1.