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ACTIVITY OF CANNABIS IN RELATION TO ITS Δ′-*trans*-TETRAHYDRO-CANNABINOL CONTENT

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1 Conditions have been worked out for a reliable estimation of the cataleptic activity of Δ′-*trans*-tetrahydrocannabinol (THC) after oral administration to mice, using the ring test over a period of 6 h.

2 By this method, the activity of cannabis herb and 5 crude fractions were measured against THC; at the same time the THC contents were determined chemically.

3 The B/C ratio (biological activity divided by chemical assay) was calculated for each. With cannabis herb the value was 3.3 and with extracts prepared with ethanol or 70% ethanol the values ranged from 3.2 to 7.1, indicating that in all samples the activity was much higher than would be expected from their THC content.

4 The cannabinoids were completely extracted from a sample of herb using petroleum spirit and the marc examined for a possible synergist. Surprisingly, it contained a powerful inhibitor of the action of THC, which could be restored by intraperitoneal prostaglandin E_2 (3 μg/kg). Some crude fractions had inhibitory activities about 10 times that of aspirin.

5 In contrast, the petroleum spirit extract (referred to in **4**) had a surprisingly high B/C ratio of 23, indicating that a powerful synergist of THC activity is present.

6 The net effect of the herb and ethanol extracts is probably due to a balance of synergist and inhibitor.

Introduction

Isbell, Gorodetzsky, Jasinski, Claussen, Spulak & Korte (1967), Jaskinski, Haertzen & Isbell (1971), Isbell (1971) and Kiplinger (1972) using human volunteers showed that the effects of smoked cannabis were similar to that of Δ′-*trans*-tetrahydrocannabinol (THC). However, they did not demonstrate what proportion of the activity could be accounted for by the THC content. Similarly in animals it has never been possible to explain all the effects of the crude material in terms of THC alone (Mechoulam, Ben-Zvi, Shani, Zemler, Levy, Edbury & Grunfeld, 1972; Carlini, Masur, Karniol & Leite, 1972). However, in both man (Isbell, 1971; Carlini, Karniol, Renault & Schuster, 1974) and in animals (Carlini *et al.*, 1974) the activity of the herb and extracts is proportional to their THC contents. In an attempt to establish the ratio of the content of THC to the biological activity we have made use of the fact that the cataleptic effect of very small doses of THC given orally can be measured in mice and that the cataleptic effect is dependent on the availability of prostaglandins E_2 (PGE_2) (Fairbairn & Pickens, 1979; 1980). We have therefore devised a reliable bioassay based on the ring test and used it to compare the activity of a suspension of the herb with extracts made with ethanol and 70% ethanol in the hope that some fractionation of the active substances would take place. The results led us to investigate further the petroleum spirit extract of the herb, followed by an aqueous extract.

Methods

Preparation of material for oral administration

Δ′-trans-*Tetrahydrocannabinol* was suspended in 2.5% solution of Tween 80 as previously described.

Cannabis Herbal cannabis was prepared by careful drying of plant material grown in our experimental gardens. A South African variety, UNC 335, was used throughout except for Sample 6 (Table 2) which was from a cannabidiol (CBD)-rich strain UNC 354 (Fairbairn & Liebmann, 1974). It is possible that the cannabinoids occur in the fresh plant almost entirely as acids but during drying and further processing, decarboxylation may take place (Doorenbos, Fetterman, Quimby & Turner, 1971) to varying extents. Since the oral activity of the acid may vary from that of the neutral cannabinoid (Korte, Haag & Claussen, 1965; Mechoulam, Ben-Zvoi, Yagnitinsky & Shani,

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1969) it was decided to decarboxylate some of our samples to the neutral form by heating at 120°C for 2 h. For administration the powdered herb was suspended in mucilage of tragacanth B.P.

Ethanol and 70% ethanol extracts were prepared by shaking 1.0 g of the powder with 20 ml solvent for 20 min, filtering and shaking the marc with a further 20 ml and filtering. The combined filtrates were reduced almost to dryness by evaporation *in vacuo* and the soft residue triturated with 0.5 ml Tween 80 and gradually suspended in water to 20 ml.

Petroleum-ether (40°–60°C) extracts were made by percolating 50 g of the powdered herb with approximately 500 ml of solvent till all traces of cannabinoid were extracted. This took about 24 h. The petrol extract was reduced to 50 ml exactly and aliquots evaporated to dryness and prepared for the mice by suspending in Tween 80, as described above.

Water extracts The petroleum spirit-exhausted marc was dried and extracted with water by percolation till 10 ml per g of marc had been collected. The extract was reduced to small bulk by evaporation *in vacuo* and transferred to a tared weighing bottle and stored in a P_2O_5 vacuum desiccator to constant weight. The marc yielded about 11% water soluble extractive.

Chemical estimation of tetrahydrocannabinol

All suspensions given to the mice were analysed for THC and CBD content by gas liquid chromatography (g.l.c.) analysis, using the method of Fairbairn & Liebmann (1973). The coefficient of variation in these assays is 1.4% for THC and 2.55% for herbal samples.

Cataleptic activity

LACA Tuck No. 1 strain albino female mice weighing 18 to 23 g were used throughout. The mice were prepared for the test by placing them in a cabinet maintained at 30 to 32°C for 3 days before carrying out the test as this reduces the coefficient of variation in the cataleptic response to THC (Fairbairn & Pickens, 1980) which was determined by the ring test and was carried out as described in our previous paper (Fairbairn & Pickens, 1979). Mice were placed 7 to a cage and all 7 received the same treatment. On each day one group was given a dose of THC, the other groups (usually two) were given doses of herbs or extacts at t_0 min. It was possible to tell by t_0 + 30 min if a suitable dose had been chosen, that is to say, whether the mice were cataleptic. If a cataleptic effect was apparent at t_0 + 30 min then the cataleptic effect of this group was also measured at t_0 + 4 and t_0 + 6 h. If, however, there was no catalepsy then the dose was increased; if there was over 75% catalepsy a lower dose was chosen. Cataleptic effects at t_0 to 1 h and t_0 + 2 h were determined in two fresh groups of mice, not necessarily on the same day. Some of the mice in these groups were also tested at t_0 + 4 and t_0 + 6 h and the scores added to those previously obtained. The same mice cannot be used at t_0 + 0.5, t_0 + 1 and t_0 + 2 h as care must be taken not to re-test any individual mouse without the minimum interval of 2 h, otherwise it would give too high a cataleptic response (Fairbairn & Pickens, 1979). We have combined all the work on the standards and extracts carried out between March 1976 and December 1979 and we have based all calculations on cumulative linear moving means of the cataleptic responses carried out on different days. An example from the responses to 0.5, 1, 3 and 9 mg/kg of THC at t_0 + 1 h will make clear what this involves and is described in detail in the results. Fresh extracts, however, were prepared immediately before testing in most experiments. In a few experiments extracts up to 6 days old were used and in these there was no loss of potency.

Inhibitory activity

Inhibitory activity was measured against a fixed dose of THC, 3 mg/kg, orally. Control mice received THC only at t_0 min; treated mice received in addition varying doses of inhibitors, administered orally also at t_0 min. The catalepsy was measured at t_0 + 1 h and the potency expressed as CDI_{50}, the dose of inhibitor in mg/kg for a 50% reduction in the cataleptic score for the control group. CDI_{50}s and their 95% confidence limits were calculated by Finney's probit analysis (Finney, 1964), from the linear graph relating probit percentage reversal of control cataleptic scores (ordinate scale) against log dose of inhibitor (abscissa scale). We have also tested aspirin as an inhibitor of THC and used it as a standard to which the inhibitory action of extracts can be compared. From the CDI_{50} values we have calculated the amount (mg) of aspirin which is equivalent to 1 mg extract.

Calculation of cataleptic potency

We have expressed potency of cataleptic compounds and extracts in two ways: as the cataleptic dose fifty (CD_{50}) which is the dose in mg/kg required for 50% catalepsy and calculated with 95% confidence limits by Finney's probit analysis (Finney, 1964), from the linear graph relating probit percentage catalepsy (ordinate scale) against log dose (abscissa scale).

Calculation of areas

We have also calculated cataleptic potency as CDA_{50} which is the dose in mg/kg required for 50% reduc-

tion in the area beneath the graph relating catalepsy (expressed as $Y_2\%$) on the ordinate scale against time to 6 h on the abscissa scale. The method of calculation of Y_2 is described in detail for 160 mg/kg of cannabis herb. The cataleptic effect for 160 mg/kg of cannabis herb as a percentage was plotted as ordinates on a linear scale against time in h (abscissa scale). The area beneath this graph was 362.25 calculated as the sum of the areas of the triangles ABC (0.25) (51) + BDE (0.25) (53−51) + DFG (0.5) (66−53), and the sum of the areas of the rectangles BCHE (0.5) (51) + DHIG (1.0) (53) + FIJK (4.0) (66) = 362.25 (Y_1). As the first reading was at t_0 + 0.5 h the maximum possible area (A_{max}) beneath the graph ALM is (600−25) = 575 and so area (Y_1) as a percentage of A_{max} was 63.00% (Y_2). In Figure 1, 63.00% was plotted on a probit scale (ordinate) against log dose (abscissa). CDA_{50} is the dose in mg/kg for 50% response in Figure 1, 95% confidence limits of the CDA_{50} were calculated by probit analysis as described above (Finney, 1964). All probit calculations were carried out by computer.

Biological/chemical ratios (B/C)

The B/C ratio for each sample is the THC equivalent by biological assay (B) divided by the THC content by chemical analysis (C).

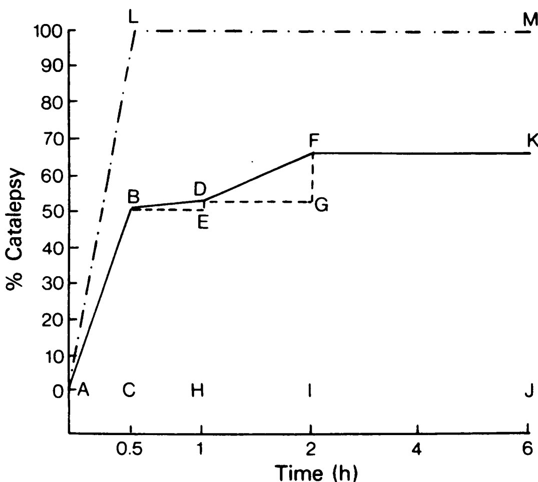

Figure 1 Overall activity of cannabis herb, 160 mg/kg, from t_0 to t_0 + 6 h. The area under the graph is the sum of the areas of the triangles ABC (0.25) (51) + BDE (0.25) (53−51) +DFG (0.5) (66−53) and the sum of the areas of the rectangles BCHE (0.5) (51) and DHIG (1.0) (53) + FIJK (4.0) (66) = 362.25 (Y_1). Area under graph ALM (A_{max}) and is equal to (600−25) = 575. Y_1 as a % of A_{max} = 100 (362.25/575) which is 63% (Y_2).

Results

Evaluation of the bioassay method

Cumulative linear moving means for all responses have been calculated in these assays and the CD_{50} values are based on the final percentage. Table 1 shows the cataleptic scores/mouse 1 h after oral administration of 3 mg/kg of THC, for 6 experiments carried out on different days, using 7 mice each day. On day 1 the mean score was 178 ± 35 and on day 2 160 ± 41. The cumulative mean for days 1 and 2 was therefore 169 ± 26. The cumulative mean for all 6 experiments was 176 ± 12 and this is the figure that has been used in the calculation of the CD_{50} for THC at t_0 + 1 h. Figure 2 shows the dose-response relationship for THC at t_0 + 1 h for 5 doses of THC. This satisfactory dose-response relationship held at all the times tested and the CD_{50} s calculated from this and other dose-response lines are given in Table 2. There was no significant deviation from parallelism for the dose-response lines for THC, herb and extracts at all the times tested. Figure 2 illustrates this for the results at t_0 + 1 h. Comparisons between THC, herb and extracts are therefore valid.

Herb and ethanol extracts

The results for cataleptic activity and chemical analy-

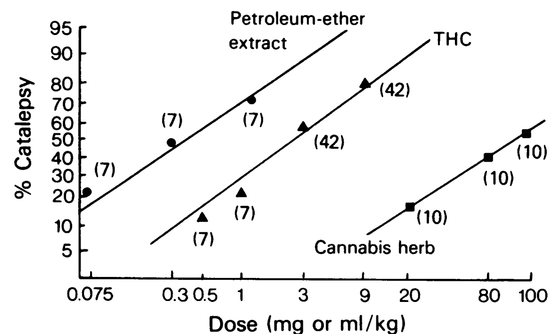

Figure 2 Relative potencies of Δ'-*trans*-tetrahydrocannabinol (THC), cannabis herb and petroleum ether extract at t_0 + 1 h after oral administration to female albino mice (Tuck No. 1 strain) at 30 to 32°C in the ring test. Ordinate scale: probit percentage catalepsy; abscissa scale: log dose mg (or ml)/kg. The number of mice injected at each dose is given in parentheses. Potency as CD_{50} for THC is 2.64(2.53–2.75) mg/kg; for herb 128 (118–140) mg/kg and for the petroleum extract 0.38 (0.35–0.41) mls/kg. The potency of the herb which contained 3.04% THC is less than would be expected due to the presence of an inhibitor. The potency of the petroleum extract which contained 1.85 mg THC/ml was greater than would be expected due to the presence of a synergist, unmasked by the removal of the inhibitor during the petroleum ether extraction.

Table 1 The cumulative linear moving mean of the cataleptic scores measured at $t_0 + 1$ h after oral administration of Δ'-*trans*-tetrahydrocannabinol (THC) to female albino mice (Tuck No. 1 strain) at 30 to 32°C in the ring test, performed on different days.

Dose THC (mg/kg)	*Date*	*Scores/mouse*	*Mean score*	*Cumulative moving mean score*
9.0	25.3.76	275, 278, 270, 292, 262, 228, 122	246 ± 22 (7)	246 ± 22 (7)
	31.8.78	290, 291, 298, 279, 232, 268, 295	279 ± 9 (7)	262 ± 12 (14)
	3.11.79	210, 203, 213, 160, 220, 191, 178	196 ± 8 (7)	240 ± 11 (21)
	9.11.79	282, 240, 164, 200, 220, 170, 184	208 ± 16 (7)	233 ± 9 (28)
	12.11.79	266, 236, 280, 160, 240, 240, 180	228 ± 16 (7)	232 ± 8 (35)
	21.12.79	278, 275, 265, 232, 216, 223, 220	244 ± 10 (7)	234 ± 7 (42)
3.0	25.3.76	187, 5, 228, 135, 260, 15, 280	178 ± 35 (7)	178 ± 35 (7)
	31.8.78	125, 0, 270, 30, 228, 217, 250	160 ± 41 (7)	169 ± 26 (14)
	2.11.79	270, 33, 226, 209, 249, 190, 5	169 ± 40 (7)	169 ± 21 (21)
	7.11.79	228, 135, 260, 150, 280, 125, 84	180 ± 28 (7)	172 ± 17 (28)
	8.11.79	234, 212, 219, 132, 220, 224, 190	204 ± 13 (7)	178 ± 14 (35)
	20.12.79	150, 160, 260, 132, 175, 178, 110	166 ± 18 (7)	176 ± 12 (42)
1.0	19.3.76	40, 46, 80, 175, 34, 30, 125	75.7 ± 21 (7)	75.7 ± 21 (7)
	21.5.77	62, 136, 31, 37, 28, 20, 38	50.3 ± 15 (7)	63.0 ± 13 (14)
0.5	10.7.78	0, 34, 102, 4, 32, 46, 23	34.4 ± 13 (7)	34.4 ± 13 (7)

Mean scores are given ± s.e.mean. No. of mice is given in parentheses.

sis have both been expressed in terms of mg THC. For convenience the B/C ratio (see Methods) for each sample is given (Table 2) and represents how much more active the crude material is compared with that expected from its THC content. For the herb the value was 3.3 with individual results at $t_0 + 0.5$, $t_0 + 1$, $t_0 + 2$, $t_0 + 4$ and $t_0 + 6$ h being respectively 1.20 ± 0.22, 0.67 ± 0.04, 1.60 ± 0.46, 5.37 ± 2.50 and 5.42 ± 1.25. These results show that the THC equivalent by bioassay exceeds the THC content by chemical analysis (3.04 mg) at all times except $t_0 + 1$ h. At $t_0 + 0.5$ h although the THC content is greater by bioassay it is not a statistically significant increase ($P > 0.05$).

The ethanol and 70% ethanolic extracts (samples 3 to 6) also showed similar high values for the B/C ratio with bioassay results exceeding those for chemical

Table 2 Potency, expressed as CD_{50} (the dose in mg/kg required to give 50% catalepsy at different times after oral administration at t_0 h) and as CDA_{50} (the dose required to give a 50% effect over 6 h) of Δ'-*trans*-tetrahydrocannabinol (THC), cannabis herb and extracts to female albino mice (Tuck No. 1 strain) at 30 to 32°C in the ring test

No.	*Sample* (THC by g.l.c.)	*Time* (h)	CD_{50} (mg/kg or ml/kg)	CDA_{50} (mg/kg or ml/kg)	*THC by bioassay* (% or mg/ml)	*B/C ratio*
1	THC	t_0 + 0.5	5.98(5.73–6.23)			
		t_0 + 1	2.64(2.53–2.75)			
		t_0 + 2	2.22(2.09–2.36)			
		t_0 + 4	8.25(7.72–8.78)			
		t_0 + 6	5.32(5.12–5.52)	6.13(5.75–6.54)		
2	Herb	t_0 + 0.5	162(149–176)			
	28–82	t_0 + 1	129(118–140)			
	(3.04%)	t_0 + 2	45(40.6–50.9)			
		t_0 + 4	50(43.7–58.4)			
		t_0 + 6	88(81.2–94.6)	60.5(53.4–68.3)	10.13	3.3
3	Ethanolic	t_0 + 0.5	9.60(9.20–10.3)			
	extract	t_0 + 1	9.36(9.23–9.51)			
	28–159	t_0 + 2	3.16(2.95–3.39)			
	(0.135 mg/ml)	t_0 + 4	6.37(4.87–7.87)			
		t_0 + 6	3.65(3.47–3.85)	6.39(6.09–6.68)	0.96	7.1
4	Ethanolic	t_0 + 0.5	8.80(8.44–9.52)			
	extract	t_0 + 1	2.16(2.36–2.80)			
	28–150	t_0 + 2	0.90(0.81–1.02)			
	Decarboxylated	t_0 + 4	1.14(1.10–1.21)			
	(1.16 mg/ml)	t_0 + 6	2.24(2.12–2.36)	1.28(1.51–1.42)	4.79	4.1
5	70% Ethanolic	t_0 + 0.5	1.09(1.04–1.20)			
	extract	t_0 + 1	0.41(0.40–0.64)			
	28–95A	t_0 + 2	0.56(0.53–0.59)			
	(2.52 mg/ml)	t_0 + 4	0.31(0.29–0.33)			
		t_0 + 6	0.52(0.49–0.60)	0.75(0.72–0.79)	8.17	3.2
6	70% Ethanolic	t_0 + 0.5	>300,000			
	extract	t_0 + 1	>300,000			
	28–95E	t_0 + 2	>300,000			
	THC — traces	t_0 + 4	>300,000		trace	
	CBD	t_0 + 6	>300,000			
	0.41 mg/ml					
7	Petroleum	t_0 + 0.5	1.20(1.14–1.26)			
	extract	t_0 + 1	0.38(0.36–0.41)			
	28–152A	t_0 + 2	0.22(0.20–0.23)			
	Decarboxylated	t_0 + 4	0.115(0.105–0.125)			
	(1.85 mg/ml)	t_0 + 6	0.076(0.070–0.083)	0.143(0.13–0.16)	42.9	23

All samples were administered orally at t_0 h and the catalepsy measured at the times shown, 95% confidence limits are given in parentheses. Herb = aqueous suspension of cannabis in mucilage of tragacanth B.P. The extracts were given in 2.5% Tween 80. Doses of THC and herb are in mg/kg and extracts in ml extracts/kg. The THC content of the herb and extracts by g.l.c. analysis is given in parentheses (column 2). Samples prepared from decarboxylated herb are noted in column 2. THC by bioassay has been calculated from CDA_{50} values (column 5).

assay at most of the times tested. Neither mucilage of tragacanth nor Tween 80, when tested alone, had any cataleptic activity.

Inhibitory and synergistic factors

As some potentiating effect seemed possible a further 70% ethanolic extract was made, evaporated to small volume and water added to precipitate resinous matter, containing the bulk of the THC. The filtered aqueous fraction was shaken with petroleum spirit to remove traces of THC present. When tested it exhibited practically no cataleptic effect; THC was therefore added to determine whether a THC synergist was present. To our surprise there was an actual reduction in the THC effect which was restored by an

intraperitoneal injection of 3 μg/kg of prostaglandin E_2 (PGE_2). This led to a search for a PGE_2 inhibitory fraction (see later). Our search for a counter-balancing powerful synergist was rewarded when a petroleum spirit extract was prepared: this had the extremely high B/C value of 23.0 and at all the times tested, the bioassay results were higher than those for the chemical assay (Table 2). Sample 6 was a 70% ethanolic extract of herb from a cannabidiol (CBD) strain which contained only traces of THC; the extract had practically no cataleptic effect. When THC was added to it the activity of the THC remained unaltered, indicating the absence of inhibiting or synergistic substances. Since the inhibitory principle was probably almost insoluble in petroleum spirit we used this solvent to exhaust a sample of cannabis herb until no more cannabinoids were extractable. The marc was dried, re-extracted with water and the aqueous extract evaporated to dryness and suitable doses given to mice; the results for 3 such extracts are given in Table 3. The first extract at 79.2 mg/kg inhibited the action of 3 mg THC/kg by 80%. We therefore repeated this dose of extract and found it inhibited 9 mg THC/kg by 51% and 27 mg THC/kg by 25%, suggesting that the inhibitor was a surmountable antagonist. By extrapolation we estimate that the CDI_{50} against 3 mg THC/kg would be approximately 13.7 mg extract/kg. Since aspirin under these conditions has a CDI_{50} of 15.5 mg/kg then 1 mg of the aqueous extract is approximately equivalent to 1.14 mg aspirin (Table 3). Results on the other two aqueous extracts confirmed this.

Two ethanol extracts were next prepared from the petroleum spirit-exhausted herb which had been further extracted with water. Both were dried to constant weight over P_2O_5 *in vacuo*; Extract 28.129A represented 1.88% of the petroleum exhausted herb; 28.131B represented 2.56%. Suitable aliquots were given to mice and the results given in Table 3 showed that these crude extracts were approximately 10 times as active as aspirin in inhibiting THC action. Apart from the results shown in Table 3, we made several other tests (21 in all, involving 301 mice) and in every one there was evidence of powerful inhibitory action.

Discussion

The purpose of our work had been to determine equally effective doses of THC (as standard) and cannabis herb and extracts (test preparations) using the ring test in mice and this is a bioassay as defined by

Table 3 Potency, expressed as CDI_{50} (the dose in mg/kg for 50% reduction in the cataleptic response at t_0 + 1 h to 3 m/kg of Δ'-*trans*-tetrahydrocannabinol (THC) orally) of aspirin and some extracts of cannabis

No	*Extract* + *THC* (3 mg/kg)	*Dose* (mg/kg orally)	*Cataleptic score*	*% inhibition of THC*	*CDI_{50}* (mg/kg)	*Aspirin (mg) ≡ 1 mg residue*
	Aspirin	40	48 ± 29 (7)	73 ± 10		
		20	73 ± 17 (7)	58 ± 6		
		15	82 ± 28 (7)	53 ± 9		
		10	116 ± 29 (7)	34 ± 10	15.52	
		5	136 ± 18 (7)	22 ± 6	(14.61–16.30)	
		0	176 ± 19 (21)	—		
1	Aqueous extract	79.2	36 ± 24 (10)	80 ± 8	ca.13.66	ca.1.14
2	Aqueous extract	44	11 ± 11 (7)	94 ± 3	c.a. 6.83	ca.2.27
3	Aqueous	99	56 ± 18 (7)	68 ± 6		
	extract	33	109 ± 23 (7)	38 ± 7	50.6	
		11	142 ± 27 (7)	19 ± 9	(47.3–53.1)	0.30
4	Ethanolic	10.3	57 ± 26 (7)	67 ± 8		
	extract	2.57	78 ± 40 (7)	54 ± 13		
	28–129A	1.03	94 ± 35 (7)	46 ± 12	1.55	
			171 ± 31 (7)	0	(1.43–1.71)	10
5	Ethanolic	21.3	35 ± 23 (7)	80 ± 7		
	extract	2.13	83 ± 28 (7)	53 ± 9	1.79	
	28–131B	0.43	121 ± 24 (7)	31 ± 8	(1.51–2.11)	8.7

Control mice received THC 3 mg/kg orally at t_0 min and treated mice received both THC 3 mg/kg and inhibitor orally also at t_2 min. Catalepsy was measured at t_0 + 1 h. The number of mice is given in parentheses in column 4; 95% confidence limits of CDI_{50} are given in parentheses.

Finney (1964a). Our estimates of cataleptic potency have been based on the cumulative moving means (Shewhart, 1931; Irwin & Cheeseman, 1939; Thompson, 1947; Thompson & Weil, 1952; Bliss, 1952; Bliss & Pabst, 1955; Cohen, Van Ramshorst & Tasman, 1959) of the cataleptic scores for control groups which were given only THC and which were assessed on different days between March 1976 and December 1979. This may appear to be contrary to the established principles of bioassay in which it is customary for each test to be self-contained and its error estimated from internal evidence, although such comparative assays have no logical basis (Gaddum, 1953) and they can be both costly and wasteful when all previous experience of a test is disregarded. Under certain circumstances, such as occur on this assay, a long series of results may be pooled in order to give increased precision (Finney, 1964c). When measuring a biological response it is usually assumed that there will be a difference in response from day to day and randomisation is planned with this in mind (Finney, 1964b). It is more difficult to plan this if it is impractical to do more than a small portion of the assay in one day, which is the case in this cataleptic work where each dose of standard and extract is measured at $t_0 + 0.5$, $t_0 + 1$, $t_0 + 2$, $t_0 + 4$ and $t_0 + 6$ h; and because of the large biological variation which accompanies cannabis work (Carlini *et al.*, 1974) and which is confirmed from our data (see Table 1) where the lowest score was zero (from a non-reacting mouse) and the highest score 270. One way of overcoming the difficulty is to study those factors which cause the 'day to day' variance and this we have done for the ring test (Fairbairn & Pickens, 1980). From this work we have shown that the three most important factors are (1) diet; (2) room temperature; (3) absence of stress; and in the work described in this paper we were careful to feed the mice the same food, to keep them at 30 to 32°C for 3 days before using them to ensure a uniformly high laboratory temperature and to reduce the stress of handling for 3 days before the test. We believe that by doing this we have reduced the day to day variance in the cataleptic responses to THC, cannabis herb and the petrol extract (Table 1), so that the variances observed are an inherent property of the reaction of the mice to THC and extracts, thereby making it justifiable to use the cumulative linear moving mean responses. This means that we have made use of all our previous data in this ring test and the final estimates of potency have been based on much larger sample sizes than would have been possible if comparisons between THC (standard) and extracts (test preparation) had been limited to data collected on the same day (or days). The data in Table 1 seems to justify this approach and shows that as the mean moves so does the s.e.mean reduce. This principle holds for all the doses of THC tested (Table 1). The method has the added advantage of reducing the daily work load in these experiments to manageable proportions. It is not generally appreciated that the 'difference between days' which is the main reason for randomising between standard and test groups which respect to the day of the experiment, is often due, as it is here, to small sample sizes in a day's work compared to the standard deviation, which is an inherent property of the data, and is not necessarily due to day to day variations *per se*.

Variation of relative potencies with time

The peak activities of THC, herb and extracts do not coincide so that potencies of these extracts relative to THC will vary according to the time at which the readings are made; the only meaningful way in which relative potencies could be calculated was to measure total activity over the 6 h period by the 'area method' described under Methods. The comparisons of potency are valid as the overall dose-response relationships do not differ significantly from parallelism (Figure 3).

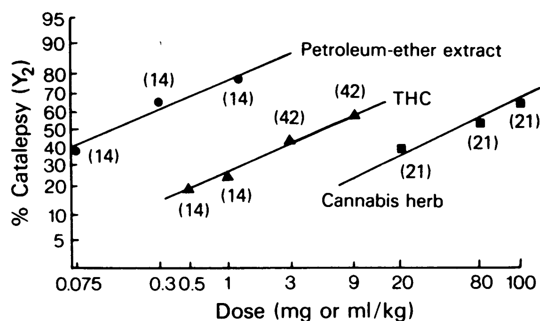

Figure 3 Relative overall potencies of Δ'-*trans*-tetrahydrocannabinol (THC), cannabis herb and a petroleum ether extract from t_0 *to* $t_0 + 6$ h, after oral administration to female albino mice (Tuck No. 1 strain) at 30 to 32°C in the ring test. Ordinate scale: probit Y_2 (see legend for Figure 1) abscissa scale: log dose mg (or ml)/kg. The number of mice injected at each dose is given in parenthesis. Potency as CDA_{50} for THC = 6.13 (5.75–6.54) mg/kg; for herb = 60.5 (53.4–68.3) mg/kg and for the petroleum extract = 0.413 (0.13–0.16) ml/kg. The potency of the herb which contained 3.04% THC is greater than would be expected as the synergist is more powerful than the inhibitor; overall B/C is 3.3. The potency of the petroleum extract which contained 1.85 mg THC/ml was greater than would be expected; overall B/C is 23.

A powerful inhibitor

The results in Table 3 indicate the presence of a powerful water and ethanol soluble inhibitor of the action of THC. Since this effect can be reversed by 3 μg/kg PGE_2 intraperitoneally, its action, like that of

aspirin and indomethacin may be involved with the biosynthesis or release of prostaglandins. The crude ethanolic extract has an inhibitory action about 10 times that of aspirin, and as it probably contains a lot of ballast, the pure substance, or substances, must have a very high inhibitory action. This substance may therefore be of considerable pharmacological interest apart from its contribution to the action of cannabis.

A powerful synergist

In spite of the presence of an inhibitor it is clear from the results in Table 2 that the herb and its preparations show activities significantly higher than expected from their THC content and this does not seem to depend on whether the THC occurs in the decarboxylated form or not. Fortunately a potential explanation lies in the fact that the petroleum spirit extract has a very high B/C ratio of 23.0. This extract contains THC but probably no inhibitor as the latter was prepared from herb from which all cannabinoids had been removed by petroleum spirit extraction. The high activity may be due to the presence of active plant constituents other than THC or to some substances which potentiates the action of THC. Sample 6, which contains traces only of THC, had practically no cataleptic effect neither did it inhibit or potentiate THC when added to it. The potentiating factor seems therefore to be closely connected with the presence of THC but it is difficult to envisage how it could exert such a powerful effect. In any event the two factors are in such proportions in the herb that the net effect is still significantly higher than that due to THC alone. Other work has also suggested that this is the case. For example, the acute lethality in tadpoles of a sample of crude cannabis could not be accounted for in terms of the THC content alone. The LD_{50} for THC was 8 μg/ml, for crude extract 14 μg/ml. Had the THC content of the extract been solely responsible, its LD_{50} would have been about 50 μg/ml, i.e. a B/C ratio of 3.6. (Paton, Pertwee & Temple, 1972), which is very close to our ratio (3.3) for cannabis herb.

Carlini *et al.* (1974) prepared three resins by petroleum-ether extraction from cannabis herbs which contained 0.82 (A), 2.02 (B) and 0.52 (C) % THC, respectively. When these resins were tested, together with THC in man (pulse rate, psychological reactions and disruption of time production task), in mouse (catalepsy and sedation), rabbits (acorneal reflex) and rat (climbing rope test), it was found that in both man and animals, resin B was twice as active as resin A and that resin C was the least active, thus confirming that activity in humans and animals runs parallel with THC content. However, they also concluded that samples A and B had activities 'two or four times greater than that expected from their THC content'. These figures agree closely with ours for the herb suspension and ethanol extracts but not for our petroleum spirit extract (Table 2) which was the solvent used by them. Calculation of the B/C ratios from their animal experiments show that for sample A they are 5.9, 6.9, 4.3 and 2.07; for sample B, 4.6, 2.5, 2.3 and 0.89; the corresponding values for sample C are 25.6, 19.6, 21.0 and 11.3 respectively. Clearly sample C values are close to our own sample 7 (Table 2) with a value of 23.0. It may be significant that their sample C, like ours, was prepared from plants of known origin, grown and prepared under controlled conditions. In contrast, samples A and B were police seizures and may well have been badly prepared and stored. The high concentrations of cannabinol (CBN) in samples A and B would support this (Fairbairn, Liebmann & Rowan 1976). Carlini *et al.* suggest that this higher content of CBN may potentiate the effects of THC; however none of our samples had more than a trace of CBN. Their other suggestion that CBD may exert an inhibitory effect is not supported by our work as we found the powerful inhibitor in plant material from which all cannabinoids were removed; furthermore sample 6 (Table 2) which contained CBD had no inhibitory effect on THC action.

Our results nevertheless are in broad agreement with those of Carlini *et al.* and indicate that THC may account for about one third of the activity of cannabis herb or resin. More importantly we have shown that certain fractions of the crude drug exhibit an inhibiting and others a potentiating effect on THC and this may have an important bearing on the types of cannabis preparation used. It is well known that 'Hash oil' is being used illicitly and that it is extremely potent. Apparently the majority of samples are prepared by extraction with ethanol and therefore will contain the inhibitor; some however are prepared using petrol and may well show powerful potentiating effects. Possibly the oriental preference for passing hash smoke through water (in which the inhibitor would dissolve) is a better way of enhancing the psychoactive effects.

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