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Structure Activity Relationship of Four Tetrahydrocannabinols and the Pharmacological Activity of Five Semi-Purified Extracts of *Cannabis sativa* *

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Abstract. The structure activity relationships of four tetrahydrocannabinols and the pharmacological activity of five semi-purified extracts from *Cannabis sativa* were studied using four biological methods: corneal areflexia in rabbits (Gayer test) and catatonia, decrease of motor activity and suppression of isolation-induced aggressiveness in mice. Modifications in the structure of pure, natural Δ^9-THC rendered the resultant compounds inactive only when activity was measured by the Gayer test; by the other three methods the activity ranged from $^1/_5$th to equal to the activity of pure Δ^9-THC. It was concluded that of the methods employed, the Gayer test was the only useful procedure to measure Δ^9-THC content in mixtures. This was confirmed by the relationship found between Δ^9-THC content and activity using five semi-purified extracts.

Key-Words: Tetrahydrocannabinol — Marihuana — Psychotomimetics — *Cannabis sativa.*

It has long been assumed that a tetrahydrocannabinol is the principle factor responsible for the effects of *Cannabis sativa* (marihuana). Among these effects are locomotor ataxia in dogs, corneal areflexia in rabbits and catatonia in small animals. Opinions, however, are not unanimous concerning this assumption. Loewe (1944) for instance, claimed that at least 3 active principles should be present in *Cannabis sativa*; Santos *et al.* (1966), on the other hand, have shown that 2 cannabis extracts of unknown chemical composition and with equal activity when tested by

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the corneal areflexia method, differed strikingly when tested on isolation-induced aggressiveness in mice. These results seem to indicate that more than one active principle is present in crude marihuana extracts. This assumption is substantiated by the fact that relatively small structural alterations of the THC-molecule cause large differences in pharmacological activity. This has been shown by the synthetic and pharmacological work of Taylor *et al.* (1967), Petrzilka *et al.* (1969) and Grunfeld and Edery (1969).

The present paper describes the pharmacological activities of four tetrahydrocannabinols and five semi-purified extracts of marihuana on the corneal reflex of rabbits, on spontaneous motor activity, on catatonia, and on isolation-induced aggressiveness in mice. Only by the measurement of corneal areflexia and catatonia was it possible to find a relationship between Δ^9-THC content and pharmacological activity.

Methods

Drugs. Extracts 1.1 TH, 1.2 TH, 1.3 TH and 1.4 TH were prepared from 3 different samples of *Cannabis sativa* confiscated by the German police. After proper chemical treatment of the samples, gas chromatography analysis was performed on the extracts revealing the chemical composition shown in Table 6. Details of the chemical procedures will be given elsewhere. Extract 2.4 TH was obtained from smoking of the plant material according to Claussen and Korte (1967). (−)Δ^9-*trans*-tetrahydrocannabinol (Δ^9-THC), Δ^{6a-10a}-tetrahydrocannabinol (Adams' THC) and 7,10,10-trimethyl-3-*n*-pentyl-1-hydroxy-6,7,8,9-tetrahydrodibenzo[a,d]-pyran (Todd's THC) were prepared by the Bonn University group (Silva *et al.*, 1968; Korte and Sieper, 1960; Claussen *et al.*, 1968). The synthesis of 3-*n*-amyl-6,6-dimethyl-1-hydroxy-1,2,3,4-tetrahydrodibenzo[b,d]-pyran (referred to as Sy-Bi) will be described elsewhere. Structural formulas of these compounds are seen Table 5.

Pharmacological Methods. All compounds and the control solution were prepared for injection according to Carlini and Kramer (1965). The Gayer test (corneal areflexia) was performed on adult male albino rabbits according to Santos *et al.* (1966); at least 5 rabbits were used with each drug. Four photocell cages, as described by van Rossum (1962), were used for spontaneous motor activity studies. The cages were modified in such a way that light beam interruptions were cumulated directly by digital counters. For each substance 4 doses in geometric progression were used; at least 10 mice were used at each dose level. All drugs were given by the intraperitoneal route and immediatly after injection the animals were placed in the cages where they remained for 3 h. To measure the catatonic state demonstrated by the mice under the drug action,

the method of Lusvarghi *et al.* (1967) was used with minor modifications. The animals had their forepaws lifted onto a horizontal glass rod which was 4.5 cm from the surface. Each animal was placed in this position 3 times, at 20 min intervals, up to 3 h after the injections. The theoretical maximum of catatonia (100%) showed by an animal should, therefore, be 10,800 sec (i.e. 3 h). Four doses, with at least 10 mice per dose, were used for each compound. The isolation-induced aggressiveness method was performed as described by Santos *et al.* (1966). A minimum of 10 mice per dose and 4 doses for each compound were analysed.

The ED_{50} necessary to reduce spontaneous motor activity, to produce catatonia and to suppress aggressiveness was calculated graphically using the log-probit method.

Results

Corneal Areflexia Method (Gayer Test). The results are shown in Table 1. Todd's THC and Sy-Bi were not able to abolish corneal reflex for doses as high as 46 and 50 mg/kg, respectively, whereas Δ^9-THC was highly active, producing areflexia with 0.114 mg/kg. Adams' THC and 2.4 TH were 42 and 263 times less active than Δ^9-THC, respectively. However, the activity of the 1.1 TH to 1.4 TH extracts correlated reasonably well with their content of Δ^9-THC.

Measure of Spontaneous Motor Activity. The results are summarized in Table 2. Δ^9-THC was the most active compound, with an ED_{50} of 18.75 mg/kg. However, a discrepancy was found when the pharmacological activity of the extracts was correlated with their content of Δ^9-THC. Thus, the effects of 1.1 TH and 1.4 TH fell within the limits expected according to their Δ^9-THC content, but 1.3 TH was almost inactive, for at 200 mg/kg it reduced motor activity to only 76.5% of control; this value lacks statistical significance. However, 1.4 TH seems to have a biphasic effect, namely decreasing activity with larger doses, but increasing it with the smaller ones. Thus, the spontaneous motor activity of mice injected with 16 mg/kg (not shown in Table 2) was 574 ± 99.

On the other hand, no correlation was found among 4 compounds when their effects were compared by the Gayer and motor activity tests. Thus, Adams' THC was 42 times less active than Δ^9-THC in Gayer test, but only 3 times less active in reducing motor activity; Sy-Bi did not abolish the corneal reflex of rabbits but significantly increased motor activity, an effect not found with any other compound. 1.3 TH had $^1/_3$ of the activity of Δ^9-THC in the rabbit, but did not significantly reduce motor activity; finally, 2.4 TH was only 10 times less active when compared to Δ^9-THC by the motor activity test.

Table 1. *Activity of 4 tetrahydrocannabinols and 5 semi-purified cannabis extracts on the corneal reflex of rabbits (Gayer test)*

Drug	Percentage of Δ^9-THC		Positive Gayer test (mg/kg ± *sd*)	Percentage of activity[a]
Δ^9-THC	pure;	100	0.114 ± 0.018	100.0
Adams' THC	pure	—	4.6 ± 1.8	2.4
Todd's THC	pure	—	46.0 (n.a.)[b]	—
Sy-Bi	pure	—	50.0 (n.a.)[b]	—
1.1 TH	impure;	27.6	0.247 ± 0.074	46.2
1.3 TH	impure;	26.5	0.327 ± 0.06	34.9
1.4 TH	impure;	9.8	1.460 ± 0.35	7.8
1.2 TH	impure;	4.6	6.451 ± 0.74	1.8
2.4 TH	impure;	0.09	34.8 ± 9.9	0.38

[a] Activity of Δ^9-THC was taken as 100%. The same for the following tables.
[b] n.a.—not active up to the dose stated. The same for the following tables.

Measure of Catatonia. The results summarized in Table 3 show that there is some correlation between Δ^9-THC content in the extracts and their catatonia-inducing properties, namely, the higher the concentration of Δ^9-THC in the extracts the higher the capacity to induce catatonia.

Todd's THC was inactive by this method, as it was with the other 2 tests. Adams' THC and 2.4 TH, however, were only 4.3 and 32 times less active than Δ^9-THC by this method, which would not be expected according to the results obtained by the Gayer test.

Suppression of Isolation-Induced Aggressiveness. Surprisingly, as seen in Table 4, Todd's THC although being inactive by the other tests, showed good activity, equal to $^1/_3$ of that of Δ^9-THC. However, Adams' THC and 2.4 TH were found to be equiactive and 2.5 times less active than Δ^9-THC, respectively, whereas with the measurement of catatonia and spontaneous motor activity they revealed activities 3 to 43 and 10 to 263 times smaller than that of the natural compound.

Discussion

According to Table 5 the main structural differences among Δ^9-THC and the 3 other compounds, are the position of the double bond, the phenolic OH group and the angular tetrahydrodibenzopyran nucleus. The importance of these chemical features in affecting pharmacological activity varies greatly according to the biological method employed. Thus, for the Gayer test the integrity of the Δ^9-THC molecule is of paramount importance, because changing the position of double bond

Table 2. *Activity of 4 tetrahydrocannabinols and 5 semi-purified cannabis extracts on the spontaneous motor activity of mice*

Drug and % of Δ^9-THC	Dosage (x in mg/kg)	Motor activity (no. of light beam interruptions) during 3 h after injection of				ED_{50} for reduction of motor activity (mg/kg)	Percentage of activity
		x	$2x$	$4x$	$8x$		
Control solution[a]	0.1 ml/10 g	408 ± 180	—	—	—	—	—
Δ^9-THC	12.5	261 ± 81	224 ± 150	184 ± 98	174 ± 78	18.75	100
Adams' THC	25.0	291 ± 239	222 ± 144	130 ± 84	81 ± 24	54.9	34.5
Todd's THC	25.0	378 ± 194	361 ± 192	382 ± 182	414 ± 35	n.a.	—
Sy-Bi	25.0	493 ± 159	558 ± 271	641 ± 270	549 ± 226	i.m.a.[b]	—
1.1 TH—27.6	8.0	391 ± 169	274 ± 71	218 ± 89	199 ± 78	46.6	40.5
1.3 TH—26.5	25.0	329 ± 130	372 ± 144	356 ± 187	320 ± 144	n.a.	—
1.4 TH— 9.8	32.0	522 ± 259	401 ± 199	196 ± 153	171 ± 113	192.0	9.7
1.2 TH— 4.6	50.0	328 ± 165	449 ± 156	309 ± 27	388 ± 101	n.a.	—
2.4 TH— 0.09	25.0	315 ± 205	315 ± 219	214 ± 79	188 ± 79	169.8	10.4

[a] 40 mice used for control solution; for all other compounds and doses, 10 mice for each were employed.
[b] i.m.a. = increased motor activity.

Table 3. *Catatonia-inducing properties of 4 tetrahydrocannabinols and 5 semi-purified cannabis extracts*

Drug and % Δ^9-THC	Dosage (x in mg/kg)	Catatonia-activity (in seconds) during 3 h after injection of:				ED_{50} for catatonia (mg/kg)	Percentage of activity
		x	$2x$	$4x$	$8x$		
Control solution[a]	0.1 ml/10 g	34.0 ± 17.1	—	—	—	—	—
Δ^9-THC	2.5	470 ± 299	970 ± 814	2230 ± 2413	3126 ± 2545	37.15	100
Adams' THC	25	317 ± 406	1785 ± 2504	4164 ± 2851	—	162.2	22.9
Todd's THC	25	37.5 ± 52.7	5.6 ± 8.7	172 ± 396	—	n.a.	—
Sy-Bi	50	—	—	17.0 ± 15.2[b]	20.7 ± 6.1[b]	n.a.	—
1.1 TH—27.6	12.5	864 ± 1020	3443 ± 3313	5095 ± 3159	5001 ± 4007	74.7	48.9
1.3 TH—26.5	12.5	378 ± 791	1755 ± 2342	2858 ± 2827	5960 ± 2424	100.0	37.1
1.4 TH— 9.8	25	482 ± 653	1010 ± 1235	2632 ± 2789	5885 ± 1038	213.8	17.3
1.2 TH— 4.6	50	123 ± 99	40 ± 34	29.8 ± 36.1	—	n.a.	—
2.4 TH— 0.09	25	291 ± 205	535 ± 225	1972 ± 1911	1809 ± 985	1200	3.1

[a] 68 mice used for control solution.

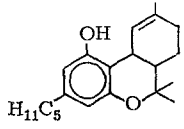
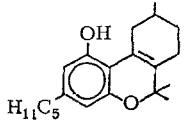
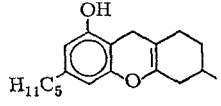
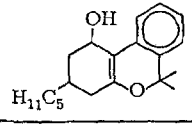
[b] Only 3 animals used. For all other compounds and doses 10 mice for each were employed.

Table 4. *Suppression of isolation-induced aggressiveness of mice by tetrahydrocannabinols and semi-purified cannabis extracts*

Drug and % of Δ^9-THC	Dosage (x in mg/kg)	Aggressiveness (seconds of fighting) after injection of:				ED_{50} for reduction of aggressiveness (mg/kg)	Percentage of activity
		x	$2x$	$4x$	$8x$		
Control solution (I)[a]	0.1 ml/10 g	24.7 ± 17.1	—	—	—	—	—
Δ^9-THC	1.25	23.5 ± 16.2	18.5 ± 9.7	8.0 ± 12.0	2.4 ± 2.6	4.25	100
Adams' THC	2.0	18.7 ± 16.4	10.6 ± 12.3	10.8 ± 7.1	5.0 ± 3.8	3.84	111.1
Todd's THC	4.0	25.3 ± 23.8	14.4 ± 20.0	7.3 ± 11.2	—	11.90	35.4
Sy-Bi	5.0	25.6 ± 20.8	19.9 ± 16.0	18.9 ± 21.2	24.4 ± 11.8	n.a.	—
Control solution (II)[a]	0.1 ml/10 g	21.8 ± 14.2	—	—	—	—	—
1.1 TH—27.6	2.0	20.9 ± 11.4	20.2 ± 18.9	12.6 ± 10.7	1.92 ± 1.53	7.80	54.6
1.4 TH— 9.8	16.0	12.3 ± 12.8	10.1 ± 3.9	2.1 ± 2.8	0.0	23.20	18.8
1.2 TH— 4.6	16.0	17.8 ± 6.4	9.4 ± 3.9	3.0 ± 4.1	0.3 ± 0.6	23.70	18.6
2.4 TH— 0.09	4.0	21.4 ± 19.8	15.7 ± 23.0	5.2 ± 9.8	3.7 ± 4.1	10.70	39.8

[a] Twelve and 30 mice were respectively for controls I and II; for all other compounds and doses at least 10 mice for each were employed.

Table 5. *Structure activity relationship among four tetrahydrocannabinols*

Compound	Structural formula	Percentage of activity in			
		Gayer test	Motor activity	Catatonia	Aggressiveness
Δ^9-THC		100	100	100	100
Adams' THC		2.4	34.5	22.9	111
Todd's THC		inactive	inactive	inactive	35.4
Sy-Bi		inacvtie	inactive	inactive	inactive

(Adams' THC) decreased activity by approximately 98%, and absence of the angular tetrahydrodibenzopyran nucleus (Todd's THC) or changing the saturation of the nucleus attached to the pyran ring (Sy-Bi), abolished pharmacological activity completely. On the other hand, the position of the double bond is not important for the induction of catatonia and the suppressive effects on motor activity, as Adams' THC was fairly active, being about $^1/_3$ the activity of Δ^9-THC. Similar results were obtained by Grunfeld and Edery (1969) in comparing the effects of Δ^1-tetrahydrocannabinol and $\Delta^{1(6)}$-tetrahydrocannabinol synthetized by Mechoulan's group. However, the absence of the angular tetrahydrodibenzopyran structure (Todd's THC) and/or changing the degree of saturation of nuclei attached to the pyran ring or phenol group (Sy-Bi) rendered the compounds inactive. Finally, Table 5 shows that suppression of the isolation-induced aggressiveness method was the least affected by changes in structure. Thus, the position of the double bond did not affect activity (Adams' THC), and Todd's THC with a linear tetrahydrodibenzopyran nucleus showed about $^1/_3$ the activity of natural, pure Δ^9-THC.

These results indicate that among the methods employed in this work, only the Gayer test is useful to measure Δ^9-THC; it was more sensitive than the catatonia method and did not give positive results for the other 3 compounds. However, its use as the only assay method for measuring the pharmacological activity of cannabis extracts, may lead to errors when attempting to obtain all active principle(s) from the plant, because a principle inactive by the former test may show good activity by any other of the 3 tests. This has previously been shown by Santos *et al.* (1966) who demonstrated that 2 extracts from *Cannabis sativa*, equiactive by Gayer's test, differed by a ratio of 16 when their pharmacological activities were measured by the aggressiveness method. These conclusions were further confirmed by the results obtained with extracts 1.1 TH to 1.4 TH (Table 6). Thus, there was a relationship between Δ^9-THC content and pharmacological activity when Gayer's Test was employed, but not so when the other 3 biological tests were employed. Among the discrepancies that arise it is interesting to analyse the failure of 1.3 TH to decrease spontaneous motor activity. According to its Δ^9-THC content such activity should be expected and failure to observe it can be explained only by accepting the existence in the extract of an antagonist which blocks Δ^9-THC action on motor activity and/or of a compound stimulating motor activity in mice which would then counteract the Δ^9-THC effects. Cannabidiol and/or cannabidiolic acid, among other substances present in the unknown components, could be such antagonistic compounds. Cannabidiolic acid can be ruled out because it is present in extract 1.4 TH, which reduced motor activity as expected

Table 6. *Correlation between chemical composition and pharmacological activity of five semi-purified cannabis extracts*

Extract	Percentage of constituents		Ratio Δ^9-THC/ cannabidiol	Percentual activity as compared to Δ^9-THC in			
				Gayer test	Motor activity	Catatonia	Aggressiveness
1.1 TH	Δ^9-THC	27.6	2.20	46.2	40.5	48.9	54.6
	Cannabidiol	12.5					
	Cannabinol	9.2					
	Cannabidiolic acid	—					
	Volatile compounds	32.8					
	Unknown	17.9					
1.3 TH	Δ^9-THC	26.5	1.27	34.9	inactive	37.1	not tested
	Cannabidiol	20.8					
	Cannabinol	—					
	Cannabidiolic acid	+ [a]					
	Volatile compounds	12.5					
	Unknown	40.2					
1.4 TH	Δ^9-THC	9.8	1.81	7.8	9.7	17.3	18.8
	Cannabidiol	5.2					
	Cannabinol	—					
	Cannabidiolic acid	+ [a]					
	Volatile compounds	25.0					
	Unknown	60.0					
1.2 TH	Δ^9-THC	4.6	1.05	1.8	inactive	inactive	18.6
	Cannabidiol	4.4					
	Cannabinol	1.0					
	Cannabidiolic acid	—					
	Volatile compounds	22.0					
	Unknown	68.0					
2.4 TH	Δ^9-THC	0.09	0.05	0.38	10.4	3.1	39.8
	Cannabidiol	1.65					
	Cannabinol	0.69					

[a] The quantitative detection of the cannabidiolic acid is not exact enough to give reliable percentage values.

by its Δ^9-THC content. On the other hand, the ratio of Δ^9-THC/cannabidiol for 1.3 TH is 1.27, whereas for extracts 1.1 TH and 1.4 TH, both effective in decreasing motor activity, the values are 2.20 and 1.81, respectively; this seems to indicate that cannabidiol could counteract Δ^9-THC effects on motor activity of mice. This suggestion is further strengthned by the fact that 1.2 TH, with a ratio of 1.05, was also inactive in reducing motor activity.

Our results also show that 1.3 TH was able to induce catatonia but failed to reduce spontaneous motor activity in mice. This discrepancy is rather apparent because decrease in motor activity although often associated with catatonia, is in reality independent from it, as has been shown by Carlini and Silva (1968). Finally, it is interesting that combustion of cannabis did not increase the pharmacological activity of the extract. Thus, 2.4 TH, in all 4 methods, had similar or lower activity as compared to extracts 1.1 TH to 1.4 TH.

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