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Cannabimimetic Activity from CP-47,497, A Derivative of 3-Phenylcyclohexanol

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ABSTRACT

CP-47,497 (*cis*-3-[2-hydroxy-4(1,1-dimethylheptyl)phenyl]-cyclohexan-1-ol) is characterized as a cannabimimetic agent, with 3 to 28 times greater potency than Δ^9-tetrahydrocannabinol Δ^9-THC), based on the following. In common with Δ^9-THC and other active structures closely related chemically to Δ^9-THC, CP-47,497 exerts analgesic, motor depressant, anticonvulsant and hypothermic effects. It elicits vocalization in palpated rats and ataxia in dogs. In drug discrimination studies in rats, the stimulus properties of Δ^9-THC (3.2 mg/kg i.p.) are generalized to CP-47,497, with an absolute threshold dose 3 to 14 times lower than the threshold dose of Δ^9-THC itself, depending on route. Furthermore, rats are unable to discriminate between the stimulus properties of equated i.p. doses of Δ^9-THC and CP-47,497 after prolonged training. Despite its potent behavioral effects, CP-47,497, like Δ^9-THC, does not resemble standard antipsychotic, antidepressant, antianxiety or hypnotic drugs in simple drug interaction tests. Based on its pharmacology, CP-47,497 exemplifies a simplified structure capable of producing many effects in common with those of Δ^9-THC and 9-normethyl-9β-OH-hexahydrocannabinol.

Δ^9-THC and active related structures exert an identifiable pattern of behavioral and physiological effects in animals, one which is distinct from that of other major classes of behaviorally active drugs. Among the best known of such effects are analgesia in opiate-responsive tests (*e.g.*, Buxbaum, 1972; *cf.* reviews in McMillan, 1977; Bhargava, 1978), decreased motor activity (McMillan, 1977), anticonvulsant activity (*cf.* Bhargava, 1978) and hypothermia (Haavik, 1977). In recent years, more specific effects have been claimed after administration of Δ^9-THC-like agents, such as a characteristic ataxia in dogs (Dewey *et al.*, 1972), vocalization on handling in rats (Henrickson and Järbe, 1971) and, even more specific, a cue in drug discrimination experiments that is not generalized to a wide variety of agents with central effects dissimilar to those of Δ^9-THC (Krimmer and Barry, 1977; Balster and Ford, 1978; Weissman, 1978). It is noteworthy that despite this readily generated cue, Δ^9-THC is a poor or ineffective reinforcer in self-administration procedures (*e.g.*, Harris *et al.*, 1974; Leite and Carlini, 1974).

In addition, Δ^9-THC differs clearly in drug interaction and other testing from the major drug classes used in psychiatric therapy. Unlike neuroleptics, for example, Δ^9-THC fails to block and may prolong or potentiate stereotypy produced by such dopaminergic agonists as amphetamine (Hattendorf *et al.*, 1977). Unlike tricyclic antidepressants, it does not counteract the actions of reserpine-like drugs (Sofia *et al.*, 1973). Unlike benzodiazepine-like antianxiety agents, its anticonvulsant efficacy is manifested prominently in animals given supramaximal ECS, but not in animals treated with pentylenetetrazol; indeed, enhancement of pentylenetetrazol convulsions may occur (Sofia *et al.*, 1976).

Based both on the distinctive behavioral effects of Δ^9-THC and on its differences from other central nervous system drugs, it is therefore possible to categorize agents as exerting Δ^9-THC-like activity independent of their chemical structure. To avoid the ambiguity of the term "cannabinoid," which is often used to signify the botanical origin of a compound or Δ^9-THC-like structure, regardless of central nervous system activity (*e.g.*, Mechoulam, 1973), we have proposed the word "cannabimimetic" as an adjective pertaining to the overall pharmacological profile produced by Δ^9-THC and particularly to its subjective effects (Weissman and Milne, 1979; Weissman, 1981). The term cannabimimetic is intended to be analogous to neuroleptic, serving to describe either a class of well defined pharmacological actions or agents, regardless of structure, that produce such actions.

The present paper describes a new 3-phenylcyclohexanol derivative, CP-47,497 (fig. 1), which appears to represent a simplified, structurally distinct cannabimimetic agent. The activity of CP-47,497 in a selection of animal tests designed to demonstrate a cannabimimetic profile is compared with that of Δ^9-THC and of racemic HHC (Wilson and May, 1975; Wilson *et al.*, 1976). This latter compound was chosen as a standard because it was the first potent Δ^9-THC analog reported to exert

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ABBREVIATIONS: Δ^9-THC, Δ^9-tetrahydrocannabinol; ECS, electroconvulsive shock; HHC, 9-normethyl-9β-hydroxy-hexahydrocannabinol; PBQ, 2-phenyl-1,4-benzoquinone; % MPE, percentage of maximal possible effect; FR, fixed ratio.

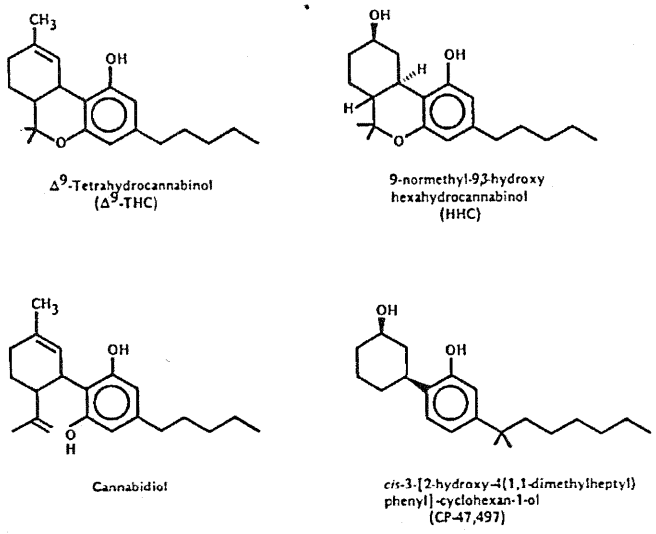

Fig. 1. Structures of cannabimimetics tested and of cannabidiol.

enhanced analgesic effects relative to its other actions and because it provided one of our starting points in an attempt to delineate minimum structural requirements for analgesic or cannabimimetic effects (*cf.* Melvin *et al.*, 1982).

Methods

Subjects. Mice used were usually Charles River males (Charles River Laboratories, Wilmington, MA), Swiss CD strain (17–21 g), fasted for 18 hr before testing. Mice in the 2-phenyl-4-benzoquinone abdominal stretching experiment were fasted Carworth males (Carworth Farms, Kingston, NY), albino CF-1 strain, weighing 11 to 15 g. Rats were Charles River males, Sprague-Dawley CD strain, weighing 180 to 220 g, unless otherwise noted. Dogs were male beagles (Marshall Farms, Wolcott, NY) weighing 8 to 12 kg.

Materials. Cannabinoid-derived agents tested and the sources of their supply were as follows: CP-47,497 (*cis*-3[2-hydroxy-4(1,1-dimethylheptyl)-phenyl]cyclohexan-1-ol; see fig. 1; synthesized by L. S. M.), Δ9-THC and cannabidiol, courtesy of National Institute on Drug Abuse (Bethesda, MD) and racemic HHC (Wilson and May, 1975; synthesized by Dr. M. R. Johnson of Pfizer Central Research, Groton, CT). Other drugs tested were donated by their respective manufacturers, as cited below, or obtained through commercial sources: pentazocine HCl (Winthrop Laboratories, Inc., New York, NY), phenytoin (Parke-Davis Division of Warner-Lambert Company, Ann Arbor, MI), diazepam and tetrabenazine (Roche Laboratories, division of Hoffmann-La Roche Inc., Nutley, NJ), *d*-amphetamine SO_4 and chlorpromazine HCl (Smith, Kline and French Laboratories, Philadelphia, PA), desipramine HCl (Geigy Pharmaceuticals, Ardsley, NJ), haloperidol (McNeil Laboratories, Inc., Fort Washington, PA), morphine SO_4, aspirin, phenobarbital Na, apomorphine HCl and PBQ (Eastman-Kodak Co., Rochester, NY).

Except where otherwise noted, CP-47,497, Δ9-THC, cannabidiol, HHC and comparative standards were dissolved and administered to rodents in a vehicle consisting of 5% ethanol, 5% Emulphor 620 and 90% saline. This vehicle alone served as the control treatment. Doses were generally based on a $\log_{10}$ dosage scale at 0.25 or 0.5 log U. Doses of salts were calculated from weights of the salt and not of the base. Routes of administration varied, as noted for each study. Solution concentrations were varied to provide a constant injection volume of 10 ml/kg of mouse and 5 ml/kg of rat.

Statistics. In several analgesic and other studies, data were first calculated as % MPE. For most studies this datum was calculated as follows:

$$\%\ \text{MPE} = \frac{\text{mean test value} - \text{mean control value}}{\text{maximum possible value} - \text{mean control value}} \times 100$$

For the PBQ study it was calculated as follows:

$$\%\ \text{MPE} = \frac{\text{mean control stretches} - \text{mean test stretches}}{\text{mean control stretches}} \times 100$$

In either case % MPE may be interpreted as the mean degree of analgesic or other effect on a given test. As it approaches 100% it indicates that the test compound produced the maximum effect possible; as it approaches 0%, it indicates that the compound produced no effect. In some studies mean % MPE data were subjected to a linear least-squares regression analysis from which an "MPE_{50}" was determined. MPE_{50} may be interpreted as the best estimate of the dose at which 50% of the maximum possible effect could be observed.

In most nonanalgesic tests, quantal dose-response data were subjected to the moving average method of Weil (1952) to give estimates of ED_{50} values.

Tests of analgesia. Blockade of abdominal stretching after PBQ test. A 0.02% aqueous solution of PBQ was prepared by dissolving 10 mg of PBQ in 2.5 ml of warmed 100% ethanol and diluting the resultant solution to 50 ml with warmed distilled water (50–60°C). The solution was retained at 40°C in a stoppered amber bottle. The selection of the Carworth CF-1 mouse strain was based on a finding that this strain exhibits particularly clear stretching.

Pretreatment times were 1 hr. Pairs of mice were then injected with 2 mg/kg of PBQ i.p. and placed in a Lucite box maintained at 40°C by a thermostatically controlled water bath. Starting 5 min later, the animals were observed for 10 min and the number of abdominal stretching responses was recorded. A stretch was defined as an intermittent contraction of the abdomen, hind limb extension, pelvic rotation or opisthotonos. Analgesic protection consisted of suppression of stretching relative to vehicle pretreated control animals tested on the same day, as described above.

Mouse tail-flick test. Tail-flick testing in mice was modified after the D'Amour and Smith (1941) procedure using heat applied to the tail. Each mouse was placed in a snug-fitting metal cylinder with the tail protruding in a slit. The cylinder was arranged so that the tail lay flat over a concealed heat lamp. At the onset of testing, an aluminum flag over the lamp was drawn back, allowing the light beam to pass through a slit and focus the heat (55°C) onto the end of the tail. Each animal was observed until it flicked its tail, in control mice usually in 2 to 3 sec, or until 10 sec elapsed.

Tail-pinch test. Rats weighing 50 to 60 g were housed in group cages of 10 animals and tested within 2 days of arrival. A 63-mm "bulldog" clamp was placed on the tail of each rat before drug treatment and again at 0.5, 1, 2 and 4 hr after treatment. The endpoint was the latency of attacking and biting behavior directed toward the offending stimulus. The trial was terminated if an attack had not occurred by 30 sec and the latency was recorded as 30 sec.

Flinch-jump test. In a modification of the flinch-jump procedure (Evans, 1961; Tenen, 1968), fasted rats were placed individually in readily viewed Lucite chambers fitted with grid floors and presented with a series of 1-sec foot shocks in increasing intensity at 30-sec intervals. Intensities were 0.26, 0.39, 0.52, 0.78, 1.05, 1.31, 1.58, 1.86, 2.13 and 2.42 mA. The behavior of each animal was rated for the presence of flinch, squeak and jump, an agitated movement of two or more limbs after shock onset. Single upward series of shock intensities were presented to each rat just before and 0.5 and 2 hr subsequent to treatment. Rats that failed to jump in response to the 0.78 mA shock intensity before receiving drug treatment were replaced. The threshold values for the jump response were recorded at each time period and % MPE and MPE_{50} values were calculated.

Rat vocalization. Vocalization was induced in fasted rats by gentle palpation of the lower abdomen (Henrikson and Järbe, 1971). Each rat was palpated five times over a period of 10 to 15 secs and the number of squeaks was recorded. Vehicle control rats produced only occasional vocalization (5 of a possible 670). Five rats were tested 1 hr after each dose and results are presented as mean squeaks over a range of s.c. administered doses.

Rotorod depression in mice. Groups of six mice were treated s.c.

and at 1, 2 and 3 hr after treatment were placed on a grooved metal cylinder of 13 mm diameter rotating at 15 rpm for 1 min and were evaluated for their performance on this rotorod according to the following scale: 0, perfect or near perfect performance; 1, riding the rotorod but with a slight difficulty; 2, riding the rotorod for about half of the 1-min period; 3, labored but mostly useless attempt to ride; and 4, total inability to remain on the rotorod. For analysis, data were dichotomized, with scores 0 or 1 denoting no effect and 2 to 4 as an effect and ED_{50} values were determined.

Motor depression in rats. Groups of six rats were tested in a cantilever-style stabilimeter with a force-displacement strain gauge. The apparatus was calibrated to record the amount of "quiescent time" during the 1- to 1.5-hr period after drug administration. The % MPE was calculated from mean percentage of quiescent time for each group, using as a base line the quiescent time of a concurrently treated vehicle control group.

Anticonvulsant tests. Mice were treated with test compounds and 1 hr later with convulsant challenges in groups of at least five per dose. ECS was administered for 0.2 sec at 50 mA, 60 Hz, through transcorneal electrodes, and mice were then observed for 10 sec for the presence or absence of hind limb tonic extension. All control mice exhibited such convulsions. Pentylenetetrazol was administered at 120 mg/kg i.p. and mice were then observed for 5 min for the presence or absence of clonic convulsions; all control mice exhibited clonic convulsions after this high dose and most controls exhibited tonic extension and mortality as well.

Interactions with amphetamine, apomorphine and tetrabenazine. Groups of five mice housed individually in Lucite cubicles were treated with CP-47,497, Δ^9-THC, HHC, selected standards or vehicle and 1 hr later with *d*-amphetamine SO_4 (20 mg/kg i.p.), apomorphine HCl (5 mg/kg i.p.) or tetrabenazine (32 mg/kg i.p.). At approximate times of peak action after these challenges (1 hr after amphetamine or tetrabenazine; 15 min after apomorphine), the mice exposed to amphetamine or apomorphine were rated for stereotypy using a 16-point scale (*cf.* Weissman *et al.*, 1966) adapted for mice and the mice exposed to tetrabenazine were rated for ptosis using the scale of Rubin *et al.* (1957) adapted for mice. The incidence of protection or reversal *vs.* the characteristic challenge-elicited symptoms was noted (scores of 0–3 in the stereotypy rating and 1 or 2 in the ptosis ratings) and ED_{50} values were obtained.

Body temperature. Groups of five mice were treated with vehicle or with five doses of CP-47,497 or Δ^9-THC and kept in a quiet room maintained at 30°C. Temperatures were measured by inserting an electronically monitored thermister 2 cm into the rectum at 1, 2, and 3 hr after treatment. Mean (±S.D.) temperature of controls at these three times were 38.1 ± 0.2, 37.9 ± 0.2 and 38.0 ± 0.3. Differences between the means of each compound-treated group and the control at each time were plotted.

Ataxia in dogs. CP-47,497 and Δ^9-THC were prepared for i.v. treatment into dogs as follows. For Δ^9-THC, 0.5 ml of a stock ethanolic solution (200 mg/ml) was dissolved in 0.5 ml of absolute ethanol; 0.5 ml of Emulphor 620 was added to this solution and then sufficient water was added to yield a cloudy suspension with a concentration of 10 mg/ml. For CP-47,497, 25 mg of compound was dissolved in 8 ml of 1,2-propanediol, to which was added 20 ml of normal saline, giving a solution containing 2.5 mg/ml. Dilutions were carried out so that i.v. injections at various doses were given at a constant volume of 0.2 ml/kg.

Behavioral effects of Δ^9-THC and CP-47,497 were graded at 15-min intervals using a modification of the scale adopted by Dewey *et al.* (1972): 0, no effect; 1, slight depression, slight ataxia, walking or running on floor, tail wagging, alert to call; 2, prance-like placement of feet, shying away from sudden movement; 3, loss of tone in back legs, hindend low to floor, tail tucked between legs; 4, swaying forward and backward and side to side but able to stand for longer than 1 min; 5, plunging about, unable to stand for 1 min without support; and 6, lying prostrate on the floor.

At least three dogs were exposed to each dose. To minimize the variability inherent in peak scores, the third value after the peak was recorded as the score. The mean values for groups of three dogs at each dose level were then subjected to regression analysis and the ED_{50} was designated as the best estimate of the dose required to produce a score of 3.

Generalization from Δ^9-THC in rats trained to discriminate Δ^9-THC from vehicle. The method used and the course of acquisition have been described in detail (Weissman, 1978). Individually housed rats received reinforcement during the conditioning procedures and sufficient supplemental chow after sessions and on weekends to maintain their weights at about 250 g. Training and testing were accomplished in operant chambers containing two levers mounted on each side of a dipper and a house-light, which remained on during the 15-min session. Reinforcement consisted of a 3-sec presentation of a commercial liquid food.

The training procedure was a two-lever FR 10 drug-discrimination protocol. Depending on whether a rat received Δ^9-THC (3.2 mg/kg i.p.) or vehicle 1 hr before the session, reinforcement was programmed exclusively on either the left or the right lever on the FR schedule. Only responses on the left lever were reinforced after drug administration and only responses on the right lever were reinforced after vehicle administration.

For drug testing, rats well trained on the above drug discrimination task were tested twice weekly with test drugs or doses other than those that comprised the training regimen. At least 12 rats were assigned to each test dose, but only data from those rats performing at a 9/10 criterion or better as of each drug test day (*cf.* Weissman, 1978) were used in calculations. As in the procedure of Colpaert *et al.* (1975), the only lever on which responses were reinforced during these 15-min test sessions was the one on which each rat first accumulated 10 responses. The major dependent variable consisted of the lever choice made by rats on test days, converted to ED_{50} values after regression analysis of the dose-effect curve. Mean response rates on both levers for the sessions also were collected as an index of general depressant properties and are presented as approximate ID_{50} values, the estimated doses that inhibited mean FR responding to 50% of mean control levels.

Although the bulk of testing for generalization from Δ^9-THC was conducted 1 hr after i.p. administration (*cf.* Weissman, 1978), an additional series of tests was performed using s.c. and p.o. routes, with a 2-hr pretreatment. Such tests were conducted to more fully explore possible selectivity differences between CP-47,497 and Δ^9-THC insofar as analgesia, determined by the rat tail pinch test, and generalization from Δ^9-THC were concerned.

A test of discriminability of CP-47,497 from Δ^9-THC. Twelve initially naive rats weighing about 220 g on arrival were used. All aspects of animal handling, apparatus and training procedure, except drug protocols, were similar to the above-described modification of the procedure of Colpaert *et al.* (1975). In the acquisition phase of this experiment, however, depending on whether an animal received CP-47,497 (0.5 mg/kg i.p.) or Δ^9-THC (3.2 mg/kg s.c.), reinforcement was programmed exclusively on the left (CP-47,497) or the right (Δ^9-THC) lever on an FR 10 schedule of reinforcement. The rationale for the selection of these doses of CP-47,497 and Δ^9-THC was based on generalization data from the preceding experiment. "Successful" discrimination would be indicated by the regular emission of 10 responses on the "correct" lever before the emission of 10 responses on the "incorrect" lever. Training was sustained for 56 days, in a reasonably concerted effort to ascertain whether or not rats could be taught to discriminate CP-47,497 from Δ^9-THC.

Results

Analgesic effects. CP-47,497 exerted analgesic effects in each procedure employed (table 1; see also table 8). The pattern of activity seen for CP-47,497 was similar to that found with morphine, Δ^9-THC and HHC, but broader than that seen after the anti-inflammatory (aspirin) and narcotic antagonist (pentazocine) analgesic standards. Although qualitatively similar to

TABLE 1
Analgesic effects of CP-47,497, Δ^9-THC, HHC, and analgesic standards in mice and rats after s.c. administration

Compound	MPE_{50} with 95% confidence limits			
	Mouse, PBQ (1 hr)	Mouse, Tail-Flick (1 hr)	Rat, Tail-Pinch (2 hr)	Rat, Flinch-Jump (2 hr)
	mg/kg s.c.			
CP-47,497	1.0 (0.35–1.6)	6.8 (2–10)	4.7 (1.3–7.2)	4.9 (2.3–7.1)
Δ^9-THC	5.9 (1.3–11.3)	55[b] (32–218)	29.1 (24.3–36.1)	83 (48–1217)
HHC	0.63 (0.26–0.97)	9.1 (5.1–21)	7.0 (4.0–16.8)	36.4 (33.3–40)
Morphine	1.8 (1.2–4.7)	5.7 (2.7–10.6)	4.8[a] (3.5–5.8)	10.3[b] (6.6–13.8)
Aspirin	116 (102–132)	>1000[c]	>1000[a]	>1000
Pentazocine	7.4[d] (1.3–12.9)	>56	>56[a]	>56[a]

[a] One-hour testing value.
[b] One-half-hour testing value.
[c] > signifies inactivity at the dose shown, the highest tested.
[d] Twenty-minute testing value.

Δ^9-THC, CP-47,497 ranged from 6 to 17 times more potent as an analgesic.

Rat vocalization. CP-47,497, like HHC and Δ^9-THC, produced vocalization in palpated rats (fig. 2) and was about 6-fold more potent than Δ^9-THC. Vocalization in rats was not seen after cannabidiol (56 mg/kg s.c.).

Decreased motor activity, sedation and operant response rates. CP-47,497, like Δ^9-THC and HHC, decreased activity in rats as measured in the stabilimeter (16 × Δ^9-THC), disrupted rotorod performance in mice (4.7 × Δ^9-THC) and inhibited response rates in the drug discrimination task (6 × Δ^9-THC) (table 2).

Anticonvulsant activity. As a 1-hr pretreatment, CP-47,497 blocked supramaximal ECS seizures with an ED_{50} of 4.2 mg/kg s.c., which is in the same range as the ED_{50} for phenytoin (table 3). Δ^9-THC and HHC also blocked tonic extension but were less potent than CP-47,497 by a factor of 23 and 3, respectively. The blockade of supramaximal ECS by CP-47,497, Δ^9-THC, HHC or phenytoin was not accompanied by blockade of pentylenetetrazol-elicited clonic seizures (table 3), although the latency to both tonic extension and mortality after the high pentylenetetrazol dose used was appreciably lengthened by high doses of these agents. Phenobarbital and diazepam, on the other hand, protected mice against clonic seizures produced by high-dose pentylenetetrazol with ED_{50} values of about 10 and 0.4 mg/kg s.c., respectively. Despite many reports to the contrary (*e.g.*, Karler *et al.*, 1973; *cf.* Bhargava, 1978), cannabidiol was repeatedly inactive *vs.* ECS at a dose of 320 mg/kg s.c. under the conditions employed

Interactions with amphetamine, apomorphine and tetrabenazine. CP-47,497 and Δ^9-THC failed to block the stereotypy caused by amphetamine or apomorphine or the ptosis and immobility caused by tetrabenazine, unlike standard neuroleptic or antidepressant agents (table 4).

Hypothermic activity. Both CP-47,497 and Δ^9-THC lowered rectal temperatures in mice, with dose-response curves that were approximately parallel (fig. 3). CP-47,497 was about 6 times more potent than Δ^9-THC. Each compound exhibited similar effects at 1 and 2 hr, but by the 3-hr point an appreciable decrement in activity had set in at the lower doses.

Dog ataxia. CP-47,497 produced ataxic effects similar to those seen after Δ^9-THC but at roughly a 4-fold lower dose (table 5).

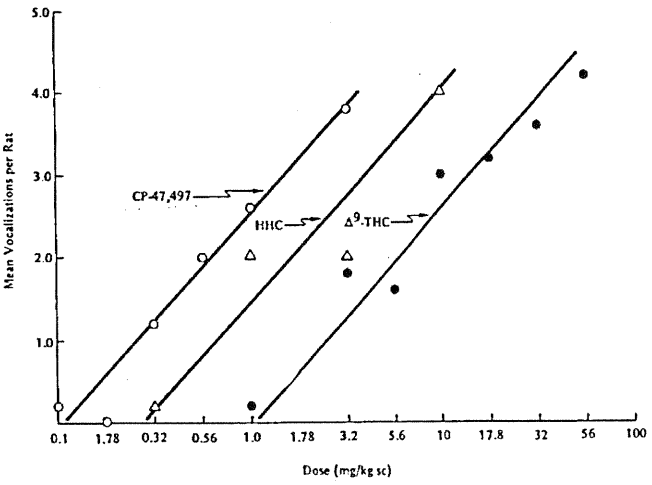

Fig. 2. Palpation-induced vocalization in rats after various doses of CP-47,497, Δ^9-THC and HHC. Each point is the mean number of vocalization responses in five consecutive trials at 2 hr after drug administration. Five naive animals were tested at each dose.

Discriminative properties. CP-47,497 was generalized by rats as similar to Δ^9-THC with an ED_{50} of 0.10 mg/kg i.p. The ED_{50} values of the training drug (Δ^9-THC itself) and of HHC were about 6 to 7 times higher (table 6).

In view of this potency difference, the acquisition experiment described under "Methods" was established with doses of 0.5 mg/kp i.p. of CP-47,497 *vs.* 3.2 mg/kg of Δ^9-THC. The results of this attempt at discrimination training are shown in table 7. It may be seen that only 1 rat (no. 179) of 12 learned to discriminate CP-47,497 from Δ^9-THC to a 10/10 criterion and that this rat was successful in maintaining a 9/10 or better performance for 55% of the trials subsequent to having first reached a 9/10 criterion. The only other rat (no. 169) that ever met at least a 9/10 criterion did not subsequently maintain the discrimination. Fully 8 of the 12 rats failed to reach even an 8/10 criterion on any of the 56 acquisition trials. It may be concluded that CP-47,497 at 0.5 mg/kg i.p. is virtually indis-

TABLE 2

Motor depressant effects of CP-47,497, Δ^9-THC and HHC in mice and rats

ID_{50}, mean response rate expressed as an index of general depressant properties. NT, not tested.

Compound	Rat Sedation Test MPE_{50}	Mouse Rotorod ED_{50} (with 95% confidence limits)			Fixed Ratio ID_{50}
	1–1.5 hr	1 hr	2 hr	3 hr	1–1.25 hr
	mg/kg i.p.		*mg/kg s.c.*		*mg/kg i.p.*
CP-47,497	1.2	0.91 (0.44–1.9)	0.72 (0.32–1.6)	1.8 (0.93–3.4)	2.2
Δ^9-THC	19.3	4.3 (2.9–6.4)	4.3 (3.1–6.0)	4.0 (2.3–6.8)	13.2
HHC	3.9	NT	NT	NT	11.0

TABLE 3

Anticonvulsant effects of CP-47,497, Δ^9-THC, HHC and anticonvulsant standards in mice: blockade of tonic extensor seizures after supramaximal ECS and clonic seizures after pentylenetetrazol

Compound	Time	ED_{50} and 95% Confidence Limits	
		Electroshock	Pentylenetetrazol
	hr	*mg/kg s.c.*	
CP-47,497	1	4.2 (2.2–9.2)	>32
Δ^9-THC	1	94.1 (71.8–127.3)	>100
HHC	1	13.3 (7.5–22.3)	>100
Cannabidiol	1	>320[a]	>320
Phenytoin	1	4.5 (3.7–6.2)	>56
Phenobarbital	3	22.1 (15.6–29.5)	10.6 (8.8–12.1)
Diazepam	1	2.4 (1.3–4.1)	0.39 (0.11–.71)

[a] > signifies inactivity at the dose shown, the highest tested.

TABLE 4

Effects of CP-47,497, Δ^9-THC and standard agents vs. amphetamine- and apomorphine-elicited stereotypy and tetrabenazine-elicited ptosis in mice

Compound[a]	Blockade of Amphetamine Stereotype (ED_{50})	Blockade of Apomorphine Stereotypy (ED_{50})	Blockade of Tetrabenazine Ptosis (ED_{50})
	mg/kg s.c.	*mg/kg s.c.*	*mg/kg s.c.*
Cp-47, 497	>32[b]	>10	>10
Δ^9-THC	>100	>100	>100
Chlorpromazine HCl	5.6 (3.2–10)	2.2 (1.6–3.0)	
Haloperidol	1.2 (0.7–2.2)	0.64 (0.4–0.9)	
Desipramine HCl			0.7 (0.5–1.2)
Pargyline			28 (17–45)

[a] All pretreatments were 1 hr except for pargyline, which was 4 hr.
[b] > signifies inactivity at the dose shown, the highest tested.

criminable from Δ^9-THC at 3.2 mg/kg i.p. Table 7, right, indicates that response rates after the two treatments during this acquisition study also were virtually identical.

Rat tail-pinch and generalization data: effect of route of administration. The rat tail-pinch data for Δ^9-THC and CP-47,497 (table 1) were extended with MPE_{50} determinations 1 hr after i.p. and 2 hr after p.o. administration. Similarly, the generalization data (table 6) were extended to include s.c. and p.o. ED_{50} determinations at times analogous to those used for analgesic testing. The combined results are presented in table 8. In all cases, the tail-pinch test was less sensitive than the generalization test and in all cases CP-47,497 was more potent,

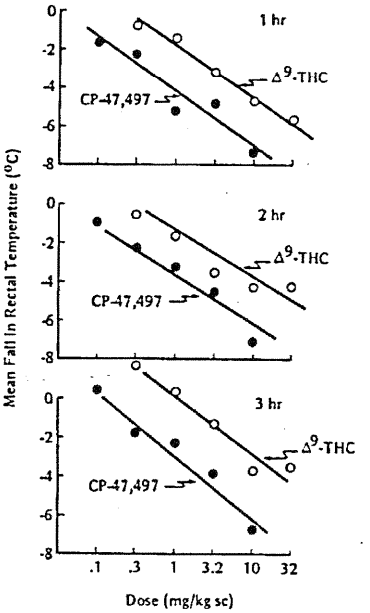

Fig. 3. Mean fall in rectal temperature of mice (N = 5 per dose) treated with CPP - 47, 497 or Δ^9-THC at 1, 2 or 3 hr subsequent to treatment. Each value was compared with a vehicle-treated control group.

TABLE 5

Ataxic effects of CP-47,497 and Δ^9-THC in male beagle dogs

Compound	Dose	*N*	Mean Ataxia Score[a] (range)	MPE_{50}
	mg/kg i.v.			
Δ^9-THC	2.0	6	5.0 (4–6)[b]	0.4
	1.0	12	4.0 (2–5)	
	0.5	6	3.2 (2–4)	
CP-47,497	0.5	6	4.3 (3–5)	0.1
	0.1	3	3.3 (3–4)	
	0.05	2	2.0 (1–3)	
HHC	0.1	3	2.5 (1–3)	

[a] Based on a modification of the symptom scale of Dewey *et al.* (1972); see "Methods."
[b] Range of scores observed for the indicated number of experimental animals.

than Δ^9-THC. By p.o. and i.p. routes, however, CP-47,497 appeared modestly more selective as an analgesic than Δ^9-THC; by the s.c. route Δ^9-THC appeared more selective.

Discussion

Despite its structural differences from prototypical cannabimimetic benzopyrans, CP-47,497 exerts a spectrum of pharma-

cologic actions qualitatively similar to that seen after Δ^9-THC and its potent analog, HHC. Some of the effects reported in this study, such as depressed rotorod performance and blockade of PBQ writhing, are generally recognized to be nonspecific and are seen after many depressant drugs. Other actions of CP-47,497, however, such as the ataxia in dogs and the vocalization in palpated rats, have been more specifically associated with cannabimimetic activity.

Perhaps the strongest single indicator of commonalty of action for CP-47,497 and Δ^9-THC is the observation that rats generalize the discriminative stimulus properties of Δ^9-THC to CP-47,497. Such generalization is seen after i.p., s.c. and p.o. treatment. An appreciable literature (for review, see Krimmer and Barry, 1977; Weissman, 1978) indicates that stimulus generalization from Δ^9-THC does not occur to a wide variety of centrally acting drugs, including standard hypnotics, analgesics, neuroleptics, anticonvulsants and anxiolytics, whereas on the other hand the drug stimulus generalization ED_{50} values of Δ^9-THC-related agents such as Δ^8-THC, 11-OH-Δ^9-THC, nabilone and cannabinol correlate well with human subjective effects (Weissman, 1978). The value of this specific, quantitative measure of cannabimimetic activity is that it makes possible the identification of new compounds that optimize potential therapeutic activity (*e.g.*, analgesia and anticonvulsant activity) relative to cannabimimetic subjective effects.

In the drug-stimulus generalization experiments cited above, the choice of the rats is between "Δ^9-THC" and "vehicle-like" stimulus effects and it could be argued tht subtle distinctions between the subjective effects of CP-47,497 and Δ^9-THC might be obscured. To test this possibility, animals were confronted with an acquisition task for 56 days (trials), requiring discrimination between Δ^9-THC and CP-47,497, and were virtually unable to learn to discriminate between pharmacologically equivalent doses of the two agents. A literature review of procedures similar to the present one (*cf.* Weissman, 1978) reveals that cocaine, diazepam, atropine, Δ^9-THC, fentanyl, apomorphine and amphetamine, among other drugs, are readily discriminated from vehicle well within 56 days. In the only previously published attempt to show that Δ^9-THC is discriminable from another centrally acting drug in an acquisition experiment, Greenberg *et al.* (1975) showed that rats readily learn to discriminate Δ^9-THC from psilocybin. The inability of rats to learn to discriminate CP-47,497 from Δ^9-THC in 56 trials strongly indicates identical discriminative stimulus properties of the two agents and not merely similar features.

The analgesic activities of Δ^9-THC (Buxbaum, 1972) and HHC (Wilson and May, 1975; Wilson *et al.*, 1976) have not been claimed to bear any relationship to subjective effects; however, activity in tail-flick and tail-pinch procedures is relatively specific for Δ^9-THC-like agents, being shared primarily with opioid-like analgesics. The potent activity of CP-47,497 on these tests

TABLE 6

ED_{50} values of i.p. administered CP-47,497, Δ^9-THC, HHC and cannabidiol for being generalized from Δ^9-THC (3.2 mg/kg i.p.) by rats

Compound	Dose	Fraction Generalizing	%	ED_{50} (95% Limits)
	mg/kg i.p.			*mg/kg i.p.*
Cp-47,497	0.018	3/12	25	
	0.032	5/12	42	
	0.056	5/12	42	
	0.1	4/12	33	
	0.18	7/12	58	
	0.32	8/12	67	
	0.56	10/12	83	
	1.0	10/12	83	
	1.8	9/10	90	0.10 (0.06–0.15)
Δ^9-THC	0.1	2/10	20	
	0.32	20/79	25	
	0.56	4/12	33	
	1.0	24/48	50	
	1.8	26/32	72	
	3.2	12/12	100	0.68 (0.38–1.2)
HHC	0.32	4/12	33	
	0.56	6/12	50	
	1.0	14/24	58	
	3.2	10/12	83	
	10.0	11/11	100	0.64 (0.51–0.78)
Cannabidiol	32.0	3/12	25	>32

TABLE 7

Acquisition data for individual rats from an effort to train them to discriminate Δ^9-THC (3.2 mg/kg i.p.) from CP-47,497 (0.5 mg/kg i.p.)

Rat	Trials to Criterion[a]			Maintenance[b] of Criterion	Mean Total Responses ± S.D.[c]	
	8/10	9/10	10/10		Δ^9-THC	CP-47,497
				%		
169	17	18	>56	2	791 ± 148	819 ± 132
170	>56	>56	>56		768 ± 49	830 ± 81
171	>56	>56	>56		602 ± 93	589 ± 231
172	>56	>56	>56		523 ± 213	533 ± 247
173	>56	>56	>56		546 ± 42	568 ± 78
174	32	>56	>56		473 ± 73	517 ± 73
175	>56	>56	>56		464 ± 124	481 ± 123
176	>56	>56	>56		769 ± 197	786 ± 244
177	>56	>56	>56		657 ± 134	624 ± 106
178	29	>56	>56		666 ± 252	685 ± 278
179	19	21	22	55	564 ± 140	533 ± 121
180	>56	>56	>56		642 ± 96	656 ± 94
Mean					623 ± 114*	635 ± 122

[a] Trials from the beginning of discrimination training until each of the noted criteria of correct choices was first reached.
[b] The percentage of trials on which at least a 9/10 criterion was maintained after the trial on which the 9/10 criterion was first met.
[c] Mean session responses during the 56 acquisition trials; 28 Δ^9-THC and 28 CP-47,497 trials. Virtually all of these responses were on the appropriate lever after the first reinforcement was received.
* $P > .10$ vs. CP-47,497; matched pair *t* test.

TABLE 8
Rat tail-pinch and generalization data for Δ^9-THC and CP-47,497 after various routes of administration

Compound	Rat Tail Pinch MPE_{50}[a]			Generalization from Δ^9-THC: ED_{50}[a,b]			"Selectivity"[c] Tail Pinch ÷ General		
	i.p. (1 hr)	s.c. (2 hr)	p.o. (2 hr)	i.p. (1 hr)	s.c. (2 hr)	p.o. (2 hr)	i.p.	s.c.	p.o.
	mg/kg			*mg/kg*					
Δ^9-THC	7.1 (4.2–11.1)	29.1 (24.3–36.1)	46.1 (14.5–70.4)	0.68 (0.38–1.2)	2.1 (1.4–2.7)	0.92 (0.61–1.3)	10.4	14.1	50.1
CP-47,497	0.59 (0.37–0.94)	4.7 (1.3–7.2)	6.4 (4.1–19.8)	0.10 (0.06–0.15)	0.16 (0.08–0.40)	0.29 (0.11–0.65)	5.9	29.4	22.1

[a] Entries in parentheses are 95% confidence limits.
[b] Fractions generalizing after each compound, dose and route were as follows: For Δ^9-THC s.c. (dose mg/kg:fraction generalization): 1.0:2/7; 3.2:4/7; 5.6:6/7; 10:7/7. For Δ^9-THC p.o.: 0.32:1/12; 0.56:5/12; 1.0:7/12; 1.8:7/12; 3.2:8/8. For CP-47,497 s.c.: 0.01:1/10; 0.032:2/9; 0.1:2/9; 0.18:4/9; 0.32:6/10; 1.0:6/6. For CP-47,497 p.o.: 0.1:3/9; 0.32:4/9; 1.0:7/9. i.p. data are given in table 6.
[c] Low numbers signify relatively greater analgetic effects.

provides another indicator of commonalty of action with Δ^9-THC.

The calculated selectivity ratios in table 8, especially after oral administration, could potentially serve as the basis for identifying CP-47,497 as therapeutically more promising than Δ^9-THC as an analgesic; however, such an interpretation must be tempered by the multiplicative error factors in the data used and by the necessarily grossly discrepant age, weight and drug experience of the rats in the two procedures. Tail-pinch testing was conducted in naive rats weighing 50 to 60 g; generalization testing was conducted in aging rats chronically maintained at 250 g and which had a considerable history of exposure to Δ^9-THC and many related agents. It is perhaps most conservative to regard the data as simply indicating route generality of the two effects and that CP-47,497 is consistently more potent than Δ^9-THC across the routes employed.

The profile of anticonvulsant activity for CP-47,497 (powerful protection against maximal ECS and no activity against pentylenetetrazol) also resembles frequently reported results after Δ^9-THC and other cannabimimetic drugs (*e.g.*, Karler *et al.*, 1973; Sofia *et al.*, 1976; *cf.* Bhargava, 1978).

That the pharmacology of CP-47,497 qualitatively resembles that of Δ^9-THC is further supported by the other tests as well. Like Δ^9-THC, CP-47,497 produces pronounced, dose-responsive hypothermia and, also like Δ^9-THC, CP-47,497 fails to block amphetamine- or apomorphine-elicited stereotypy or tetrabenazine-elicited ptosis in mice.

CP-47,497, a synthetic 3-phenylcyclohexanol derivative, differs in several key structural respects from Δ^9-THC and related structures heretofore classified chemically as cannabinoids (fig. 1). It totally lacks the pyran ring; it is not a fused tricyclic structure; as in HHC, the 9-CH_3 substituent of Δ^9-THC is replaced by an hydroxyl; the double bond of Δ^9-THC is absent. Despite these differences, molecular mechanics calculations predict that CP-47,497 adopts a low energy conformation that is nearly superimposable on the structure of HHC (Melvin *et al.*, 1982; L. S. Melvin, M. R. Johnson, C. A. Harbert, G. M. Milne and A. Weissman, manuscript in preparation). This achieves nearly perfect overlap of the putative points of receptor binding (alcohol hydroxyl, phenol hydroxyl and alkyl side chain) for these two molecules. Consistent with this, the pharmacological profile of CP-47,497, including its subjective effects, appears qualitatively similar to that of Δ^9-THC and HHC, except for varying increments in potency depending on endpoint. CP-47,497 thus illustrates our previous contention (Weissman and Milne, 1979; Weissman, 1981) that rational classification of drugs in this area should be based unambiguously on their pharmacology and not on presupposed chemical specifications or botanical origin.

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