RECENT ADVANCES IN THE USE OF CANNABINOIDS AS THERAPEUTIC AGENTS

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I. INTRODUCTION

The search for therapeutically active cannabinoids is going on in numerous laboratories throughout the world. The main thrust has been in trying to dissociate the undesirable CNS side effects (typical THC-type effects) from the potentially useful antiemetic, antiglaucoma, anticonvulsive and analgetic effects (1-3).

The main psychotropic component of hashish and marijuana, delta-1-tetrahydrocannabinol (THC) (or delta-9-THC by an alternative nomenclature), which we isolated, identified and synthesized in the sixties (4), is already in wide experimental clinical use as an antiemetic during cancer chemotherapy. However, the side effects caused by THC prevent its use in other pharmaceutical areas at present.

In the last few years we have devoted considerable amount of effort towards dissociating, by molecular modification, the CNS side effects from the anti-convulsive, antiglaucoma and analgetic effects in this series. We wish to report now on some of our results. A few of these have been disclosed in patents.

II. PHARMACOLOGICAL STUDIES

A. Anticonvulsant Activity

The anticonvulsant action of natural (-)-CBD in animals and in man is well documented (5-8). It seemed of interest to determine the structure-activity relationships in these series in order to explore the possibility of increasing the potency

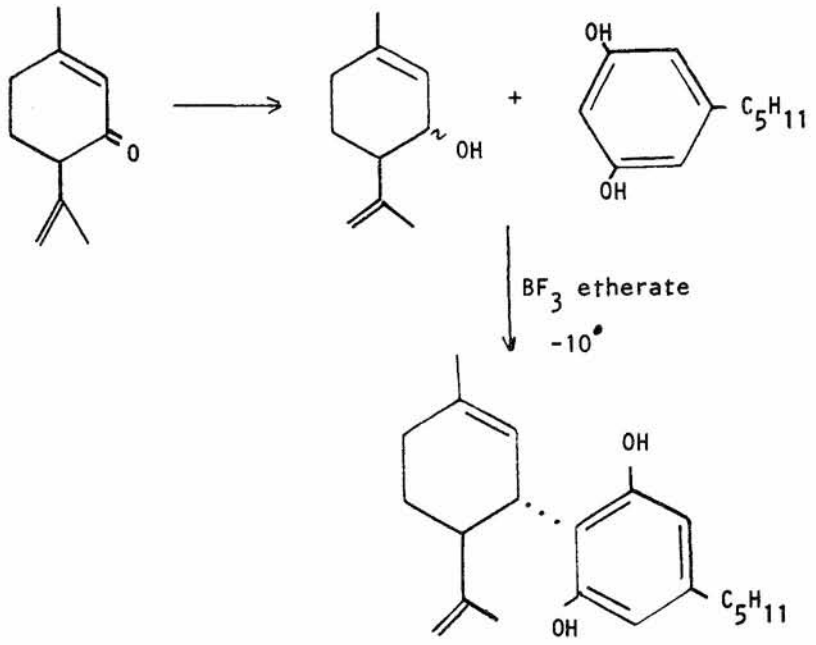
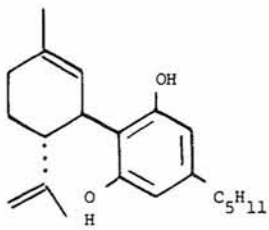


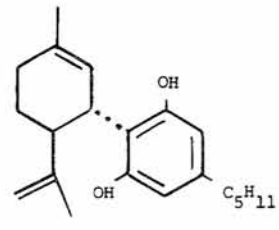
FIGURE 1. Synthesis of (+)-CBD.

as well as to obtain initial clues as to the mechanism and site of action. Some years ago, we reported on the activity of several simple derivatives of CBD (6). Most derivatives were active in the transcorneal electroshock test at approximately the same dose levels as CBD itself.

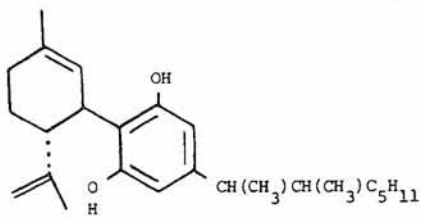
It is generally believed that the unique stereospecificity seen in biological systems is due to a complementary three dimensional interaction between a drug and an assymetric receptor site (9). The actual equivalence of activity of CBD and its analogs observed by us previously (6) led us to suspect that CBD acts on a system which does not involve a receptor site, but rather a less specialized system. In order to verify this hypothesis we synthesized the (+)-enantiomer of the active natural (-)-CBD. We assumed that a high stereospecificity in the action of CBD could indicate the existence of a three dimensional receptor or site of action. The synthetic route is described in Fig. 1. The method used is applicable to both enantiomers as the starting material is available in



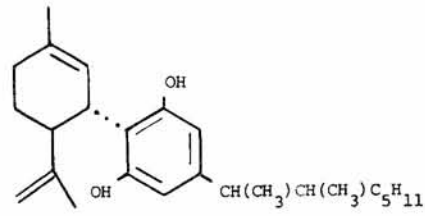
natural (-) cannabidiol
(-)CBD



(+)CBD



(-)CBD-DMH



(+)CBD-DMH

FIGURE 2. CBD and analogs tested in the anticonvulsant test described in the text and in the legend to Fig. 3.

both the 4S and the 4R isomers. In addition, the side-chain 1,2-dimethylheptyl (DMH) homologs of both enantiomers were prepared (Fig. 2). This particular modification was chosen as in the tetrahydrocannabinol (THC) series as such a chemical change was known to greatly increase the psychotropic potency.

1. Maximal Electroconvulsive Seizures (MES). After convulsive current 99.9% (C.C 99.9%) was established, groups of ten animals were injected i.p. with the cannabinoid suspensions. At several time intervals after the injection, the animals were submitted to the test. It consisted in applying a convulsive current transcorneally. The time of hindlimbs extension and frontlimbs flexion ratio were recorded and expressed as to ratio extension/flexion (6).

2. Pentobarbitone Sleeping-Time. Groups of 10 animals each were injected with suspensions of the cannabinoids or with saline solution. Forty five minutes later, the animals received a pentobarbitone solution, 40 mg/kg, i.p. The interval between loss and recovery of righting reflex was recorded as being the sleeping-time.

3. Results. Fig. 3 shows that the (+)- and (-)-isomers of CBD are essentially equally active in the MES test. The DMH homologs of both isomers are also essentially equipotent, although they are somewhat more active than the CBD isomers.

The two CBD isomers, as well as the two CBD-DMH isomers, potentiated pentobarbitone sleeping time. At some dose levels the (+)-isomers were somewhat more potent.

The above results tend to support our assumption that CBD does not act on a specific receptor, but rather on a less specialized system.

Some years ago Banerjee et al. (10) showed that CBD inhibited the rate of cerebral cortex synaptosomal uptake of GABA. It should be a considerable challenge to correlate this finding with the apparent lack of stereospecificity of CBDaction reported now.

B. Dissociation of the Psychotropic and Analgetic Effects in a Synthetic Cannabinoid

Delta-1-tetrahydrocannabinol (THC) and several of its synthetic analogs have been found to cause analgesia in animals and man (for formula of THC and analogs, see Fig. 4). As cannabis does not have a high addiction potential, an analgetic drug based on the cannabinoid skeleton can be of pharmaceutical importance, if dissociation between the psychotropic and analgetic effects is achieved as discussed above. Indica-

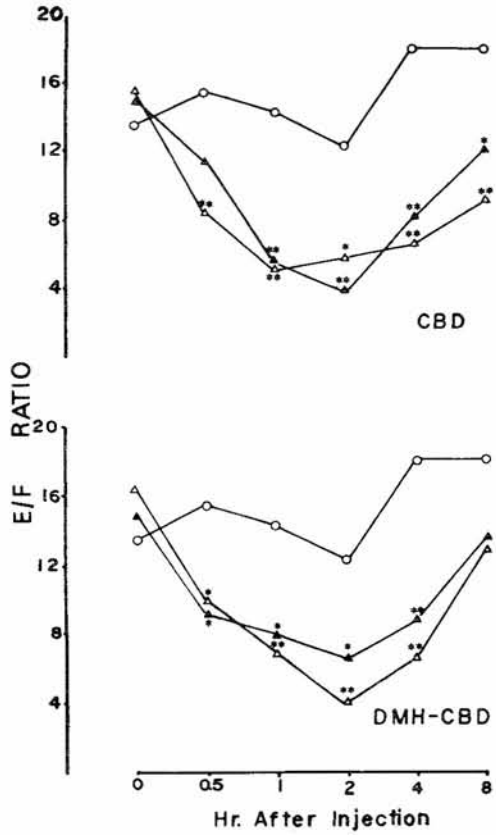


FIGURE 3. Influence of dextro (open triangles) and levo (solid triangles) isomers of CBD (100 mg/kg) and DMH-CBD (50 mg/kg) on the extension of flexion time ratio (E/F ratio). Control mice injected with the vehicle are represented by open circles. (* $p < 0.05$; ** $p < 0.01$; Student's t-test)

tions that these two effects are inherently separable have been published (11).

As part of a program aimed at achieving such a dissociation we have synthesized and tested a series of cannabinoids. The complete results will be published elsewhere. We wish to report now that in preliminary pharmacological tests the THC analog I has been found to have approximately retained the analgesic activity of the parent drug but to be at least 20 times less psychotropic than THC (12).

The most salient feature of the new analog I is that its two chiral centers (at C-3 and C-4) have a configuration oppo-

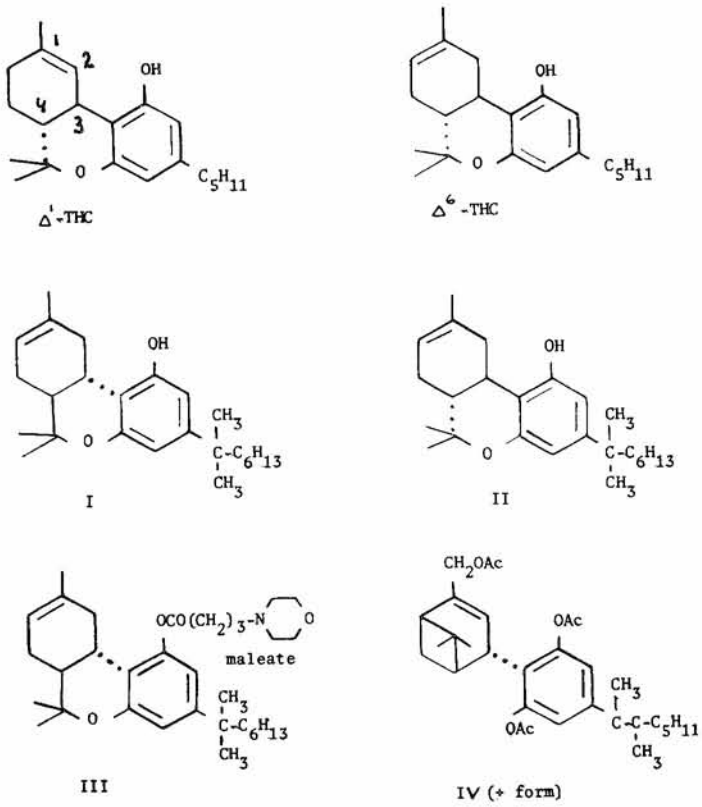


FIGURE 4. Delta-1-THC and analogs.

site to that found in the natural cannabinoids, i.e., they are 3S,4S rather than 3R,4R as in THC. The optical rotation of I is hence essentially identical (but opposite in sign) to that of the enantiomer II, a cannabinoid with very high psychotropic potency.

1. Synthesis. We have followed the general route previously developed by us (4b) for 3S,4S cannabinoids (Fig. 5). The optical purity of I (and consequently the level of psychotropic activity) obviously depends on the optical purity of the starting material (+)-pinene from which verbenol is obtained. In our best preparation, starting from (+)-pinene (a) + 51.4°, we obtained I, $[a]_D$ + 218°. Enantiomer II, in which the chiral centers correspond to those in the natural series showed $[a]_D$-2122°.

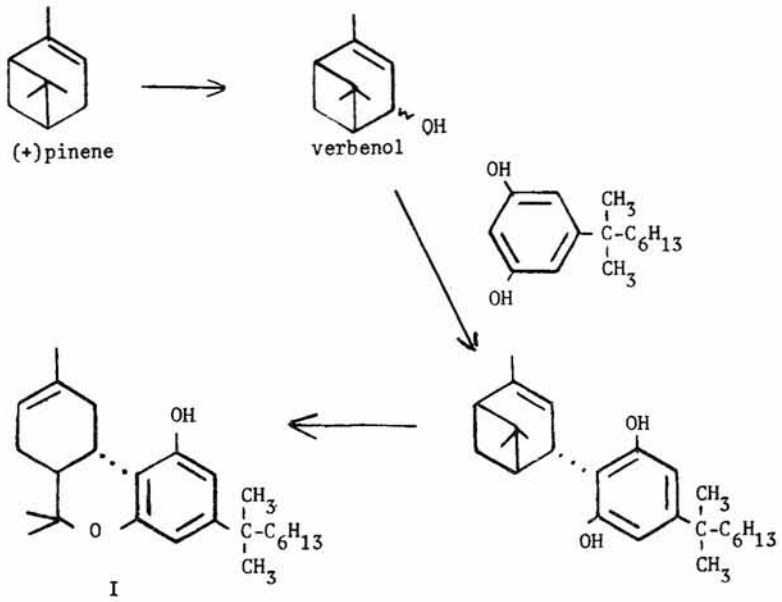


FIGURE 5. Synthesis of THC analog I.

2. Analgetic tests. All compounds were administeredas fresh suspension (0.1-0.2 ml) orally to mice. The test compound was dissolved in propylene glycol and a solution of 2% gun accacia was gradually added to form the suspension. The final concentration of propylene glycol was never above 5%. The tests were carried out at least on 3 dose levels in order to calculate the dose response lines.

a. Acetic acid induced writhing test. Six mice perdose level were examined. Thirty minutes after administration of the drug 0.6% acetic acid (0.25 ml) was injected i.p. The numberof abdominal contractions per animal was counted for 25 minutes (13).

b. Tail flick test. Ten mice per dose level was examined. The mouse was held in the standard receptacle. The tail was immersed into water at 58°C and immediately withdrawn on jerk. If no response occurred up to 5 sec., the tail was withdrawn. After a 5 sec. rest the procedure was repeated for a total of

10 times. The percentage of negative responses were noted and statistically evaluated. We found that counting the negative responses gave more reproducible data than measuring the time of the response as the original paper (14).

3. Psychotropic Tests. The psychotropic activity of cannabinoids has been tested in a number of laboratory animals including dogs, mice, rats, gerbils and monkeys. A comparison of the major somatic and behavioral effects by a THC in man and in rhesus monkey shows a reasonable similarity: close threshold effective doses (ca. 50 mcg/kg), dose dependent effects, impairment of motor coordination, redness of conjuctivae, pseudoptosis, loss of muscle strength, heart rate increase, decline of aggression, sleepy state, impairment of performance, etc. Some of these symptoms such as redness of conjunctivae and pseudoptosis, seem to be specific for primates. Hence the rhesus monkey test has been found the most suitable (15).

The compounds were administered i.v.; the solvent used was propylene glycol. Injections were made into the saphenous vein at a maximum volume of 0.1 ml/kg. The observers were unaware of the nature of the compounds being tested. At least two animals were used for the testing of each compound at every dose. The estimation of the psychotropic effects is semiquantitative. The effects were monitored and rated as previously described (16).

C. Results and Discussion

A significant difference between the enantiomers I and II is observed (see Table I). The (-)-enantiomer II, in which the chiral centers correspond to those of the natural cannabinoids, causes a cataleptoid state in mice at the analgetic dose range of THC, thus preventing any meaningful evaluation of an analgetic dose response curve. The analgetic effect of the (+)-enantiomer I in both tests is at the range of THC and delta-6-THC; its dose response lines do not significantly deviate from the parallel lines of morphine. The (+)-enantiomer I is ca 20 times less psychotropic than THC; the (-)-enantiomer II is more potent than THC.

A point of interest is that the analgetic activity of cannabinoids administered orally is better manifested in the writhing pain test than in the tail test. As writhing pain is assumed to be mediated by prostaglandin formation, it seems possible that the analgetic effect reported now is due to this inhibition (18).

It must be taken into account, however, that the optical purity of I depends on the optical purity of the starting

TABLE I. Mouse Analgesia and Monkey Behavioral Tests[a]

Compound	Mouse ED_{50}[b]		Monkey Psychotropic Activity	
	Writhing	Tail Flick	mg/kg	rating
Delta-1-THC	5	50	0.05	(+/-)
			0.10	(++)
			0.25	(++)
			0.50	(+++)
Delta-6-THC	7	>80	0.1-0.3	(+)
			0.4-0.9	(++)
			1.0-2.0	(+++)
Compound I	9	60	1.0	(-)
			2.0	(+)
			4.0	(++)
Compound II	Pc	P	0.02	(-)
			0.05	(+)
			0.10	(+++)
			0.25	(+++)[d]
Compound IV			1.0	(-)
+ isomer	10	50	4.0	(-)
- isomer	10	30	5.0	(-)
Morphine	4	20		

[a]For experimental conditions see text.

[b]Expressed in mg/kg and calculated according to Lichfield and Wilcoxon (17). The dose-response curves did not deviate from parallel.

[c]P, denoted psychotropic activity, due to which analgesia could not be determined.

[d]The behavioral changes were remarkably intense and lasted for three days.

material which is seldom absolute. Hence a low percentage (1-2%) of II may be present in the tested samples of I. A possible pharmacological interaction between I and II cannot be excluded.

The observation that the analgetic and psychotropic activity are separable may be of considerable importance in the development of new analgetics.

III. NOVEL CANNABINOID ANTIGLAUCOMA AGENTS

Interest in using cannabis for the treatment of glaucoma was first stimulated by the observation of Hepler et al. (19) that intraocular pressure decreased when healthy human subjects smoked cannabis (see ref. 20 for extensive reviews). A considerable amount of research has been published on this topic. Smoking or ingestion of marijuana or THC definitely causes reduction of intraocular pressure in both healthy humans and those with glaucoma. However, intraocular administration has not led to definite results and consistent effects have not been observed (21).

Ideally a preparation that could be applied topically to the eye would be most desirable for humans because this would allow for self-administration and would limit the action to the eye. One would also look for cannabinoids which do not cause CNS side effects.

We assumed that THC showed inconsistent results when administered topically due to the extremely low water solubility. Hence we decided to prepare water soluble derivative of cannabinoids which showed no 'hashish' effects when administered to monkeys. In part 'A' of this presentation, we described the synthesis of (+)-delta-6-THC-DMH (I) and presented data showing that it has a low 'hashish' effect on monkeys. Hence we decided to prepare its water soluble maleate salt III, and test it for antiglaucoma effect. The synthesis is straightforward from compound I and follows related preparations by Pars et al. (22).

TABLE II. Reduction of Intraocular Pressure in Glaucomatic Rabbits Treated with Compound III*

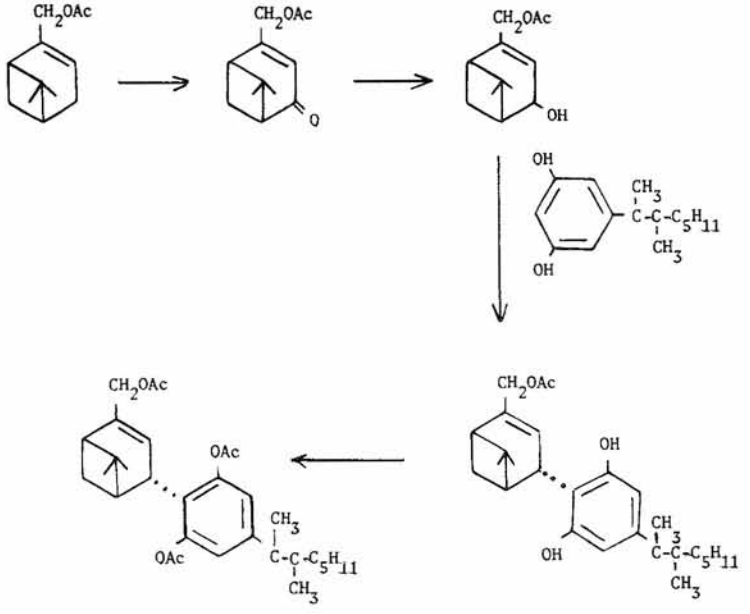
Time of Treatment	Intraocular Pressure (mm Hg)
0	29
15 min	18.5
30 min	19.5
60 min	22.0
90 min	21.5
6 hr	22
22 hr	24

*For description of method see text and ref. 23.

The maleate salt III was tested by topical application into an eye of a glaucomatic rabbit of 0.2 ml of a 0.3% aqueous solution. The glaucoma was induced by administration of Betsovet (Glaxo, betamethasone adamantoate and betamethasone sodium phosphate) as described by Bonomiet al. (23). The results are presented in Table II.

We assumed that the water soluble ester III is rapidly hydrolyzed to compound I when absorbed into the eye and is not washed away by the tears due to its lipid solubility. We expect this to be the case in humans as well, and as compound I has very low 'hashish' type activity we expect no CNS side effects.

We have demonstrated here that dissociation can be achieved between the antiglaucoma and typical 'THC-like' effects and, as in the case of analgesia, we believe that this observation may lead to the development of new antiglaucoma drugs.



IV (+ form)

FIGURE 6. Synthesis of cannabinoid IV, lacking a pyran ring.

D. Is a Pyran Ring Needed for Cannabinoid Analgetic Activity?

As mentioned above, numerous THC-type cannabinoids have been found to possess analgetic activity. All these cannabinoids possess a pyran ring. It seemed of possible interest to find cannabinoids in which major structural changes were made and yet have retained some of their pharmacological properties. We wish to report now that compounds such as acetate IV, which do not possess a pyran ring and retain the analgetic properties of THC yet show no THC-type psychotropic activity. These observations have been reported in a patent (24).

The synthesis of compound IV is described in Fig. 6. The results of the analgetic tests and the psychotropic test in monkeys are given in Table I.

Compound IV [in both the (+) and the (-) form] is about as active as THC in the writhing test but is at least 50 times less active than THC in the psychotropic test.

The observation that a pyran ring is not an absolute requirement for analgetic activity (although it may be one for THC-type activity) is of considerable importance and may lead to the eventual development of clinically useful analgetics.

E. THC Augments Opioid Activity

Both cannabis, in its various forms, and opium are widely used in Indian traditional medicine. Frequently they are used together suggesting a possible synergism. The potentiation of morphine activity by crude cannabis has indeed been documented (25). It seemed of interest to find out whether a synergistic

TABLE III. Analgesia Produced by Analgetics Administered Orally[a]

Compound	ED_{50} (mg/kg)[b]
Morphine hydrochloride	4
Codeine phosphate	17
Acetyl salicylic acid	330
(-)-delta-1-THC	5
(-)-delta-6-THC	7
Phenylbutazone	180

[a]For experimental conditions see text.
[b]Calculated according to ref. 17.

effect does indeed exist between cannabinoids and opiates. The existence of such an effect could eventually make possible the use of lower doses of opiates than those in a clinical setting, generally administered today, thus avoiding side effects.

We would like to report now on the effects of THC and delta-6-THC on various analgetics.

The acetic acid writhing test was employed as described above. Initially we tested the potency of several analgetics administered orally. The results are presented in Table III and are compatible to those in the literature.

Then we administered mixtures containing ED_{20} doses of THC and the appropriate analgetic. We found that ED_{50} doses of the analgetic tested were reduced 4-6 fold (Table IV). These results are better expressed in graphic form in Fig. 7 and 8.

These results show that the potency of certain analgetics, including opiates, can be increased considerably by the addition of low amounts of either delta-1- or delta-6-THC. These observations, if valid for man, can be of considerable importance. Thus, if the increase in potency of codeine is not paralleled by an increase in dependence liability (which for codeine is much lower than that of morphine), this relatively safe compound may find a new, very important place in the clinic. The same applies to phenylbutazone. The possibility of using doses of morphine in the clinic lower than those generally employed today is also an intriguing one.

Is it possible to augment analgetics using non-psychotropic cannabinoids? We do not have a definite answer yet but preliminary evidence is encouraging.

TABLE IV. Potentiation of Action Analgetic Agents by Delta-1- or 6-THC[a]

Compound A	Compound A[b]	Compound B[c]	Synergism
Acetylsalicylic acid	50	1-THC	6-fold
Codeine phosphate	3	1-THC	5-fold
Codeine phosphate	3	6-THC	5-fold
Morphine HCl	1	1-THC	4-fold
Phenylbutazone	40	1-THC	5-fold
Phenylbutazone	40	6-THC	5-fold

[a]For method employed see text.
[b]Dose, ED_{50} in mg/kg.
[c]Dose, ED_{20} = 3 mg/kg.

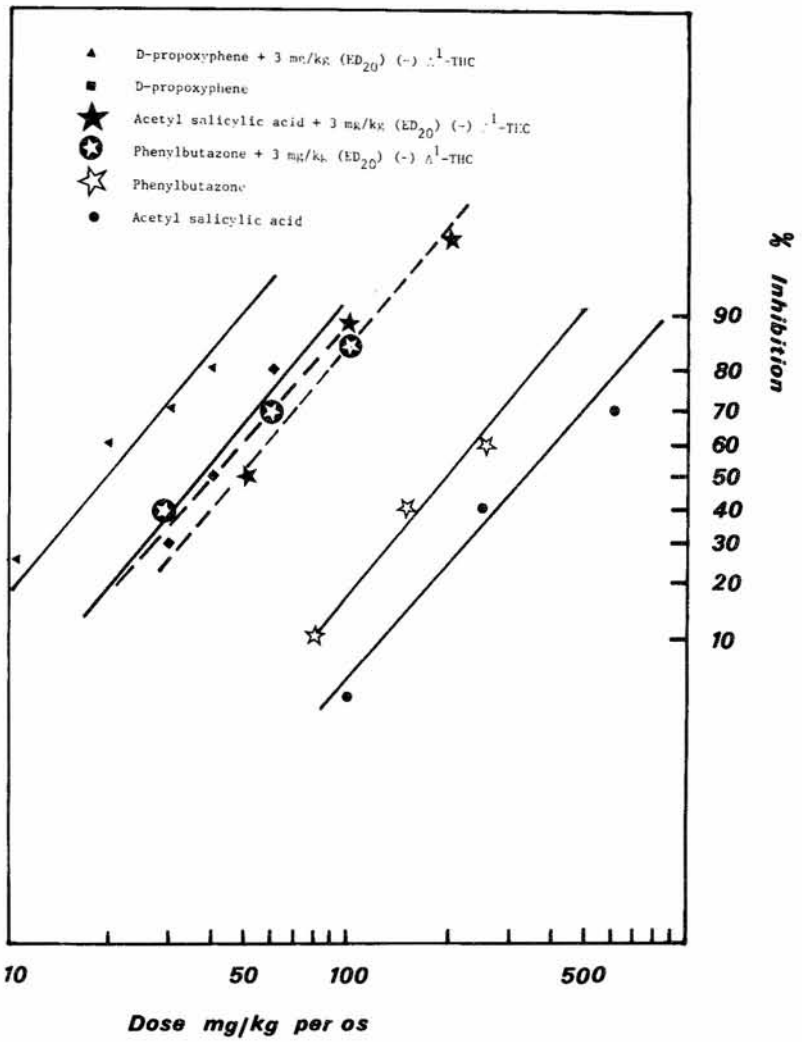


FIGURE 7. Potentiation of non opiate analgetics by THC. See text for details.

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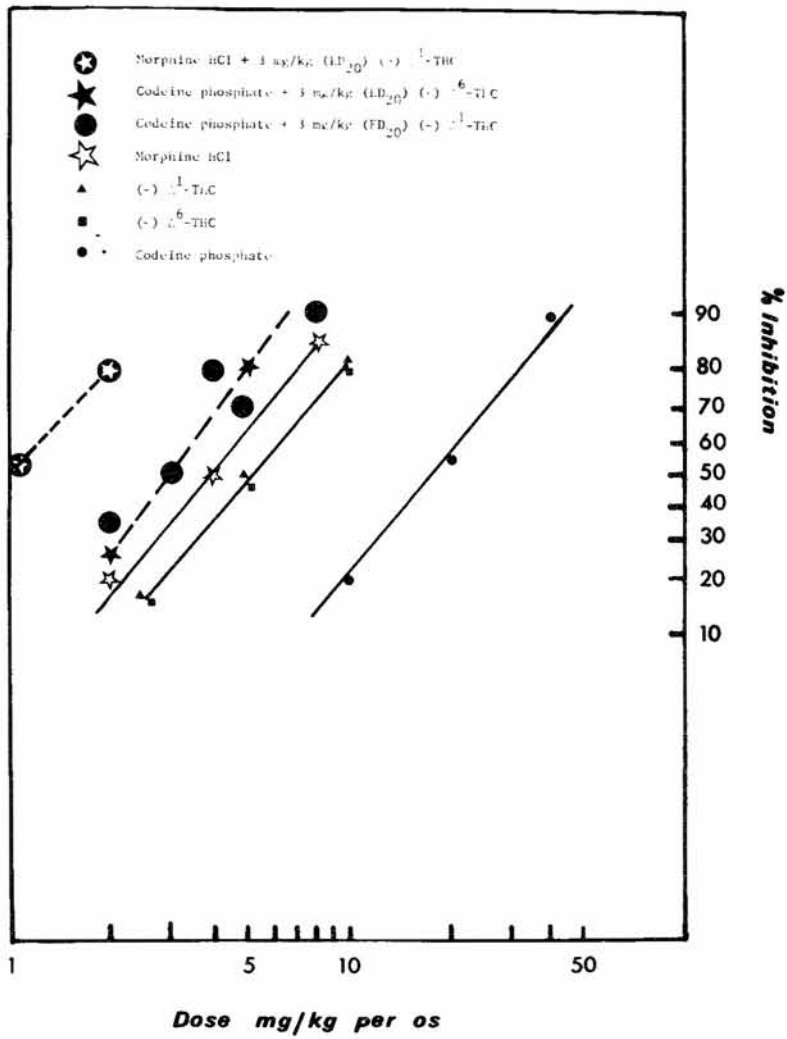

FIGURE 8. Potentiation of opiate analgetics by delta-1- and -6-THC. See text for details.

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