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A NOVEL CLASS OF POTENT TETRAHYDROCANNABINOLS (THCS): 2'-YNE-Δ^8- AND Δ^9-THCS

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Summary

A series of 3-alkyl-2'-yne (side chain) acetylenic analogs of Δ^9-THC were synthesized and evaluated for *in vitro* and *in vivo* activity. Analogs were evaluated for receptor affinity in a [^{3}H]CP-55,940 displacement assay and for *in vivo* pharmacological activity in a mouse procedure utilizing a tetrad of measures. These compounds represent a preliminary exploration of the consequences of restricting the flexibility of the side chain regarding cannabimimetic activity. All analogs proved to have receptor affinities (4-11 nM) that were five to ten times greater than that observed for Δ^9-THC. However, the *in vivo* activities of these compounds varied greatly. All analogs proved to possess the greatest potency for production of antinociception, with activity similar to or less than that observed for the production of hypomotility, hypothermia, and catalepsy. The most potent analog **11b** exhibited an ED_{50} of 0.031 mg/kg in the tail-flick procedure, with values in other measures being between 0.5 and 1.0 mg/kg. The least active compound (**11c**), though still possessing a K_I of 11 nM, exhibited ED_{50} values of 3.1 and 9.3 mg/kg for tail-flick and temperature procedures, as well as 41 and 48 mg/kg for ring-immobility and spontaneous locomotor activity, respectively. This profile (high receptor affinity but low *in vivo* potency) would normally be suggestive of a compound with antagonist properties (at least for immobility and activity measures). It is unclear why these acetylenic analogs were so potent *in vitro*, while only one (**11b**) exhibited the degree of *in vivo* potency anticipated based upon comparison to values for Δ^9-THC. It is possible these side chain modifications do not interfere with receptor recognition, but limit receptor activation or second messenger signal transduction. Regardless, it is clear these novel analogs provide a basis for the further exploration of the cannabinoid receptor pharmacophore.

Key Words: 2'-yne-Δ^9-tetrahydrocannabinols, cannabinoid receptor, structure-activity relationship

The widespread illicit use of marijuana accompanied by the interesting pharmacological profile it exhibits (1) has resulted in an intensive study into the cannabinoid field in the last three decades. Especially significant progress has been made in recent years with the identification (2) and cloning (3) of the cannabinoid receptor as well as the isolation (4, 5) of endogenous ligands. Although a great deal is known concerning the pharmacological effects of cannabinoids, their mechanism of action remains somewhat elusive (1, 6h, 6i).

The major psychoactive constituent of cannabis, Δ^9-THC, **1**, produces a unique behavioral syndrome whose effects include depression, ataxia, psychoactivity, analgesia, and cardiovascular

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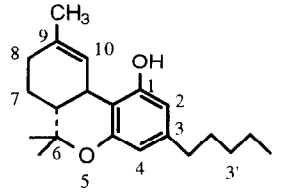
effects among others (1, 6h, 6i). To screen compounds for cannabimimetic activity, Martin *et al.* (8) have developed a tetrad of tests, namely locomotor activity, hypothermia, analgesia, and catalepsy in mice, and have shown excellent correlation between these effects and cannabimimetic activity, particularly the psychoactivity of Δ^9-THC. In addition, an excellent correlation between receptor binding and *in vivo* activities of cannabimimetics has been established (9).

On the basis of SAR and molecular modeling of cannabinoids (10) it has been speculated that the important areas for receptor recognition and activation are the C-9 position, the phenolic group at C-1, and the side chain at C-3. Using molecular modeling techniques we have developed a cannabinoid pharmacophore (10d) which has shown an excellent correlation between the predicted and actual biological activity for over fifty cannabinoids whose structures vary in these three positions (8a). These effects have been shown to correlate well with animal studies.

The hydrophobic pocket in the pharmacophore, where the side chain at C3 resides, appears to be the most important in terms of cannabinoid potency and recognition. That this side chain is important was first shown in the work of Adams (7b), where the dimethylheptyl analog was found to be a hundred times more active than the synhexyl analog. In addition, recent work from our laboratories (11) has shown that in the 3'-hydroxylated THCs, the *S* isomer was found to be ten times more potent than the *R* isomer, whereas the latter was equiactive with Δ^9-THC. This suggests that an important stereochemical site of interaction is present in the hydrophobic pocket of the receptor where the side chain at C3 resides.

During the development of this pharmacophore, no data for THCs bearing rigid side chains were included. This is significant in that, when the THC molecule is at the receptor site, the actual orientation of this nonrigid moiety is unknown. One could envision this side chain adopting any number of conformations, but the particular conformation required for optimum activity is not at all obvious. We therefore embarked upon the study of THC analogs which possess a rigid side chain so as to further refine the model for the cannabinoid receptor. The introduction of a site of unsaturation would certainly impose a significant degree of rigidity upon the side chain, restricting the conformations it could readily attain and thereby leading to variations in the biological response elicited by such species. Previously only two THC analogs bearing an unsaturated side chain have been reported, both of which show increased activity relative to THC analogs with saturated side chains: these are analogs **2** reported by Loev (12) and analog **3** from our laboratories (13). Such analogs were not explored further, however, in spite of their increased potency. Hence the objective of this research was to prepare THC analogs carrying an acetylenic group in the side chain at various positions and investigate their biological activity. The number of carbons was also to be varied so as to find the optimum length, as potency had already been determined to be a function of chain length in other analogs (7b). The 2'-position was chosen initially for synthetic simplicity, and work is currently underway to obtain samples with an acetylene bond in other positions. It is hoped that the data obtained from the study of such analogs will contribute to the molecular model and further refine the pharmacophore.



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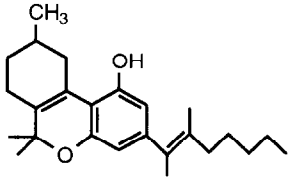

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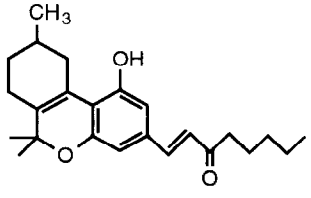

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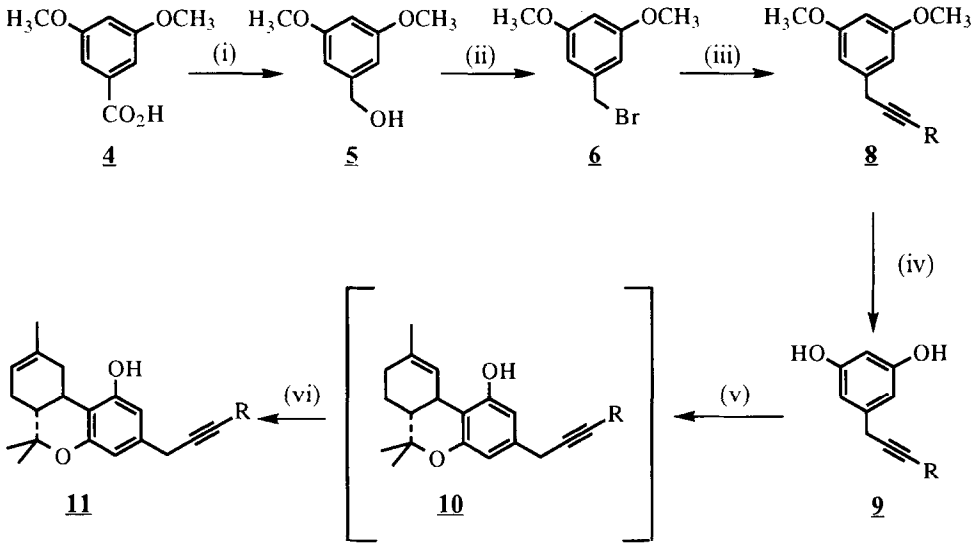
Methods

Synthesis:

The chemistry of THC analogs has been well studied (6, 7, 14). The synthesis of the acetylene-containing analogs is shown in Figure 1, and follows procedures that have previously been developed in these laboratories (14). In general the preparation involved an acid-catalyzed condensation between the appropriate resorcinol **9** and *para*-menthene-1,8-diol (14). The initially-formed Δ^9-THC analog **10** is in most cases not isolated, as further heating readily leads to isomerization to the thermodynamically more stable Δ^8 isomer.

The resorcinols were synthesized according to Figure 1 from 3,5-dimethoxybenzyl bromide **6**. This material was prepared *via* established methods: borane reduction (15) of 3,5-dimethoxybenzoic acid **4** proceeded readily to give excellent yields of 3,5-dimethoxybenzyl alcohol **5**, which was subsequently converted (16) to its corresponding bromide **6**. Pure materials were obtained in good yields by chromatography on silica gel using 10% ethyl acetate/hexanes as eluent.



$R = (CH_2)_nCH_3$

a, n = 5	**8a**, 85%	**9a**, 83%	**11a**, 20%
b, n = 4	**8b**, 77%	**9c**, 57%	**11b**, 22%
c, n = 2	**8c**, 75%	**9d**, 45%	**11c**, 30%

Fig. 1
Preparation of Δ^8 and Δ^9-Tetrahydrocannabinols

(i) BH_3/THF, reflux, 2 hr; (ii) CBr_4, tri-*n*-octylphosphine, ether, room temperature, 2 hr; (iii) $LiC{\equiv}C(CH_2)_nCH_3$ **7**, LiI, THF, reflux, 1.5 hr; (iv) BBr_3/CH_2Cl_2, -78 °C to room temp., 3 hr; (v) *para*-menthene-1,8-diol, *p*-TSA, benzene, reflux; (vi) continued reflux (2 hr total).

The 3,5-dimethoxybenzyl bromide **6** readily undergoes substitution with a variety of nucleophiles. In this particular case the nucleophile was the acetylide anion **7a-c**, prepared by treating the commercially-available alkyne with *n*-butyllithium in cold THF. This acetylide anion reacts with **6** in the presence of lithium iodide to give the desired products **8a-c** (17). Chromatographic separation on silica gel using 2% ethyl acetate/hexanes as the eluent afforded the products shown with the yields indicated.

The methoxy-protected resorcinols so obtained were deprotected (18) using BBr_3 and, after chromatographic separation on silica gel using 30% ethyl acetate/hexane, resulted in yields of resorcinol **9** ranging from fair to good. Condensation with *para*-menthene-1,8-diol then gave the desired THCs. The low yields obtained were not unexpected as it is characteristic of this THC synthesis to form a variety of side products in sometimes significant quantities. Analog **10a** was obtained by prematurely halting the reaction between **9a** and *para*-menthene-1,8-diol, and then separating **10a** from its Δ^8 isomer **11a** chromatographically. All THC products were purified by chromatography on silica gel using 5% ethyl acetate/hexanes, and the purities checked by capillary GC and elemental analysis (C,H). Proton NMR spectra consistent with the proposed structures were obtained for all compounds.

Pharmacology:

Evaluation of compounds for *in vivo* and *in vitro* activity was performed as described (8, 9) with minor modifications. Male ICR mice (Harlan, Dublin, VA) weighing 18-25 gm were maintained on a 14:10 hr light:dark cycle with free access to food and water. Δ^9-THC was obtained from the National Institute on Drug Abuse. Compounds were dissolved in 1:1:18 (emulphor-ethanol-saline) for tail-vein administration at a volume of 0.1 ml/10 g of body weight. Emulphor (EL-620, a polyoxyethylated vegetable oil, GAF Corporation, Linden, NJ) is currently available as Alkmulphor. Mice were acclimated in the evaluation room overnight without interruption of food and water. Following drug administration each animal was tested for effects on the following tetrad of procedures: spontaneous (locomotor) activity at 5 min, tail-flick latency response at 20 min, rectal temperature at 60 min, and catalepsy (ring-immobility) at 90 min. NIH guidelines for proper treatment of animal subjects were followed in all cases.

Spontaneous Activity. Mice were placed into individual activity cages (6.5 x 11 in) 5 min post-injection, and interruptions of the photocell beams (16 beams per chamber) were recorded for a 10-min period using a Digiscan Animal Activity Monitor (Omnitech Electronics Inc., Columbus, OH). Activity in the chamber was expressed as the % Inhibition *versus* the vehicle controls.

Hypothermia. Baseline rectal temperatures were determined prior to drug or vehicle injection with a telethermometer (Yellow Springs Instrument Co., Yellow Springs, OH) and a thermistor probe inserted to 25 mm. Rectal temperatures were measured again 60 min after the injection. The difference between pre- and post-injection values were calculated for each animal as Δ°C.

Antinociception. Antinociception was assessed using the tail-flick procedure (19, 20). The heat lamp of the tail-flick apparatus was maintained at an intensity sufficient to produce control latencies of 2 to 3 sec. Control values for each animal were determined prior to drug administration. Mice were then re-tested 20 min following drug injection and latencies to tail-flick response were recorded. A 10-sec maximum was imposed to prevent tissue damage. The degree of antinociception was expressed as the % MPE which was calculated as:

$$\% \text{ MPE} = \left[\frac{\text{(test latency - control latency)}}{\text{(10 sec - test latency)}} \right] \times 100$$

Catalepsy. Catalepsy was determined by a modification of the ring immobility test (21). At 90 min post-injection, mice were placed on a ring (5.5 cm in diameter) that was attached to a stand at a height of 16 cm. The amount of time (sec) that the mouse spent motionless during a 5-min test

session was recorded. The criterion for immobility was the absence of all voluntary movements (excluding respiration, but including whisker movement). The immobility index was calculated as:

$$\% \text{ IMMOBILITY} = \left[\frac{\text{amount of time immobile}}{\text{length of test session}}\right] \times 100$$

Mice that fell or actively jumped from the ring were allowed 5 such escapes. Following the fifth escape, the test for that animal was terminated and immobility was calculated as a percentage of time that it remained on the ring before being discontinued. Data from mice failing to remain on the ring at least 2.5 min were not included.

Receptor Binding. [^{3}H]CP-55,940 (K_D = 690 nM) binding to P_2 membranes was conducted as described elsewhere (9), except whole rat brain (rather than cortex only) was used. Displacement curves were generated by incubating drugs with 1 nM of [^{3}H]CP-55,940. The assays were performed in triplicate, and the results represent the combined data from three individual experiments.

Data Analysis. Statistical analysis of all *in vivo* data was performed using ANOVA with Dunnett's t-test for comparison to control. ED_{50} values were determined from least-squares unweighted linear regression analysis of the log dose-response plots. Maximal effects for all compounds combined on spontaneous activity, temperature, antinociception, and catalepsy were, respectively, 90% inhibition, minus 5 °C, 100% MPE, and 60% immobility. Thus, the ED_{50} values indicate response levels of 45% inhibition, minus 2.5 degrees, 50% MPE, and 30% immobility. The K_I values were determined from displacement data using EBDA (Equilibrium Binding Data Analysis; Biosoft, Milltown, NJ).

Results

Data for Δ^9-THC are included in the Table for comparison only (8). Compound **11c** was evaluated at 6 doses varying from 1 to 100 mg/kg. Linear correlations (r value) for the tetrad of measures were greater than 0.90, with the exception of temperature (r = 0.75) which proved to be somewhat erratic. Despite the fact that this compound bound to the receptor with great affinity, it was found to be approximately as potent as Δ^9-THC in antinociception, somewhat weaker in temperature, and 30 to 40 times less potent in the two remaining measures. The *in vivo* potencies varied from 3.1 to 48 mg/kg.

Compound **11b** was evaluated at seven doses varying from 0.01 to 10 mg/kg. Linear correlations for the tetrad of measures were greater than 0.92, with the exception of motor activity (where r = 0.83). This compound bound to the receptor with great affinity and was the most potent of the series *in vivo*, though generally only slightly more potent *in vivo* than Δ^9-THC. The in vivo potencies varied from 0.031 to 1.0 mg/kg. Interestingly, this analog was 45-times more potent in the production of antinociception than Δ^9-THC.

Compound **11a** was evaluated at six doses varying from 0.1 to 30 mg/kg. Linear correlations for the tetrad of measures varied from 0.86 to 0.92. This compound bound to the receptor with high affinity and was almost identical *in vitro* to **11b**. However, *in vivo* this analog was similar to Δ^9-THC in terms of potency, with the exception of antinociception. The *in vivo* potencies varied from 0.15 to 2.4 mg/kg. In contrast to that observed with **11b** on the tail-flick procedure (45-times more potent), **11a** was only ten times more potent than Δ^9-THC.

Compound **10a**, the Δ^9-isomer of **11a**, was evaluated at five doses varying from 0.1 to 30 mg/kg. Linear correlations for the tetrad of measures varied from 0.98 to 0.99. This compound also bound to the receptor with an affinity almost identical to **11c**, being only slightly less than its **11a** conformer. However, *in vivo* this analog was not similar to either **11a** or **11c**, but instead similar to Δ^9-THC in terms of potency. In fact, **10a** was only slightly less potent than Δ^9-THC in temperature and ring-immobility measures. The *in vivo* potencies varied from 1.4 to 4.9 mg/kg.

TABLE

ED_{50} values (mg/kg) for the *in vivo* mouse tetrad and receptor affinity from *in vitro* displacement assays (K_I, nM ±SEM).

compound	Spontaneous Activity	Rectal Temperature	Tail-Flick Latency	Ring Immobility	Binding (K_I)
1[a]	1.0[a]	1.4[a]	1.4[a]	1.4[a]	53[a]
11a	2.4	1.4	0.15	1.7	4 ± 1
11b	0.50	1.0	0.031	0.56	5 ± 2
11c	48	9.3	3.1	41	11 ± 1
10a	1.9	3.9	1.4	4.9	11 ± 4

[a]Previously published data (8,9).

Discussion

The series of Δ^8- and Δ^9-THC analogs presented herein were found to possess receptor affinities five to ten times greater than that of Δ^9-THC. All analogs were more effective in the production of antinociception than hypomotility, hypothermia, or catalepsy. However, the separation of activity was not sufficient to term these analogs selective antinociceptive agents. The greatest degree of separation of pharmacological effects existed for compound **11b**, which was 16 times more potent in the production of antinociception than for hypomotility. While it is unlikely that this differential could be exploited therapeutically, this analog does provide a viable avenue for further research in this area.

Compound **11c** essentially represents the conversion of the traditional pentyl side chain of THC to a hex-2'-yne side chain. This alteration resulted in a receptor affinity five times greater than that of Δ^9-THC *in vitro*, but was a compound less potent *in vivo*. Perhaps the relatively weak *in vivo* potency was due to pharmacokinetics. However, not all observations could be accounted for by this explanation. In fact, compound **11c** is 13- to 15-times less potent in the production of spontaneous activity and ring-immobility measures than it is for antinociception. The very weak potency of **11c** (in two measures), combined with such high receptor affinity, would normally be suggestive of an antagonist or mixed function agonist-antagonist. This possibility has not yet been explored.

Despite the rather high receptor affinities of these acetylenic analogs, only one (**11b**) exhibited the degree of *in vivo* potency anticipated based upon comparison to values for Δ^9-THC. Perhaps the hex-2'-yne side chain did not interfere with receptor recognition, thus allowing for high affinity of all analogs, but failed to allow full receptor activation or second messenger signal transduction, which could explain low *in vivo* potency of most compounds. These compounds have not been evaluated for inhibition of adenylyl cyclase or other second messenger properties.

Conclusion

The synthesis of a new class of THC analogs bearing an acetylenic bond in the 2' position has been developed and their biological activity investigated; at least one (**11c**) may be showing partial antagonistic behavior. These acetylenic analogs also indicate the importance of the side chain in the production of antinociception, which corroborates earlier findings in the non-classical series of cannabinoids of which CP-55,940 is the prototype (22). The data obtained from these studies will be used to further refine the model for the cannabinoid receptor. This model will then be used to predict the behavior of other, as-yet unsynthesized THC analogs.

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