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Investigation of the Role of the Phenolic Hydroxyl in Cannabinoid Activity

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SUMMARY

Structure-activity relationship studies have suggested that the phenolic hydroxyl group is essential for the pharmacological activity of the cannabinoids. However, it remains to be established whether it is the hydrogen of the phenolic hydroxyl that is important (possibly because this hydrogen can participate in a hydrogen bonding interaction) or whether it is the oxygen of the phenolic hydroxyl that is important (possibly because one of the lone pairs of electrons on this oxygen can serve as a hydrogen bond acceptor). Two new etherified cannabinoids were prepared in which the phenolic hydroxyl oxygen is incorporated into a fourth ring. These new compounds were designed to test the importance both of the phenolic hydroxyl oxygen and of the orientation of its lone pairs of electrons for cannabinoid pharmacological activity. *O*,2-Propano-Δ^8-tetrahydrocannabinol (0,2-Propano-Δ^8-THC) was designed to mimic Δ^9-THC in its phenol conformation I (C2-C1-O-H = 7°). *O*,10-Methano-Δ^9-tetrahydrocannabinol (0,10-Methano-Δ^9-THC) was designed to mimic Δ^9-THC in its phenol conformation II (C2-C1-O-H = 167°). Molecular mechanics calculations revealed that 1) there are two accessible minimum energy conformers for *O*,2-propano-Δ^8-THC, which differ principally in the conformation of the new fourth ring, and 2) there are three accessible minimum energy conformers for *O*,10-methano-Δ^9-THC, the first two of which differ mainly in the conformation of the new fourth ring, whereas the third possesses an alternate pyran ring conformation. Wave functions and molecular electrostatic potential (MEP) maps were calculated for each accessible conformer of *O*,2-propano-Δ^8-THC and of *O*,10-methano-Δ^9-THC. The resultant MEP maps compared well with the corresponding MEP maps generated for Δ^9-THC in each of its two minimum energy conformations (two phenolic hydroxyl positions). These results imply that 1) *O*,2-propano-Δ^8-THC should be capable of being recognized at a site that would recognize Δ^9-THC in its phenol conformation I and 2) *O*,10-methano-Δ^9-THC should be capable of being recognized at a site that would recognize Δ^9-THC in its phenol conformation II. Pharmacological evaluation of the analogs revealed that *O*,10-methano-Δ^9-THC was inactive in all mouse tests, as well as the rat drug discrimination model. *O*,2-Propano-Δ^8-THC was similar to Δ^8-THC in that it depressed rectal temperature and produced antinociception and ring immobility in mice. However, it differed from Δ^8-THC in that it only weakly depressed locomotor activity and failed to substitute for Δ^9-THC in the drug discrimination paradigm. A similar separation of cannabinoid pharmacological effects has not been possible heretofore. These results suggest that the orientation of the lone pairs of electrons on the phenolic hydroxyl oxygen plays an important role in the mediation of some, but not all, behavioral effects of the cannabinoids.

The cannabinoids are the group of C_{21} compounds present in *Cannabis sativa L.* plus their biotransformation products and synthetic analogs (1). A surge of scientific interest followed the reports of Gaoni and Mechoulam (2, 3) that identified Δ^9-THC (Fig. 1) as the major psychoactive component of cannabis. The site or sites of action of the cannabinoids are as yet unknown. However, a cannabinoid binding site in rat brain has recently been characterized and has been proposed to be a THC receptor (4).

The SAR for the pharmacological activity of the cannabinoids has been generated based upon whole-animal models (see Ref. 5 for review). One of the traditional conclusions drawn regarding the SAR of the cannabinoids is that the position of and the environment around the phenolic hydroxyl are critical for activity. Specifically, a free phenolic hydroxyl at position C1 is believed to be necessary for pharmacological activity. Esterification of the phenolic hydroxyl does not eliminate activity. However, it can be argued that esterified cannabinoids can be hydrolyzed *in vivo*, resulting in the free phenolic hydroxyl. On the other hand, etherification of the phenolic hydroxyl eliminates activity. The conclusions reached concerning etherified cannabinoids are based primarily on early studies of *O*-methyl-Δ^9-THC and *O*-methyl-Δ^8-THC in rhesus monkeys

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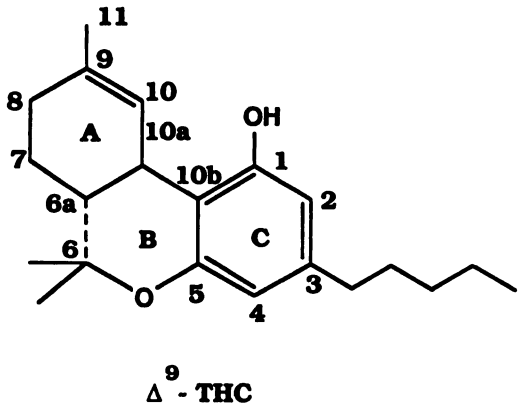
ABBREVIATIONS: Δ^9-THC, (−)-(*trans*)-Δ^9-tetrahydrocannabinol, Δ^8-THC, (−)-(*trans*)-Δ^8-tetrahydrocannabinol; FR, fixed ratio; MEP, molecular electrostatic potential; MPE, maximum possible effect; SAR, structure-activity relationship; CHFEP, coreless Hartree-Fock effective potential.



Δ^9 - THC

Fig. 1. One numbering system commonly employed for Δ^9-THC.

(6) and dogs (7). These two etherified compounds were found to be inactive in rhesus monkey behavioral assays and 1/25th as active as Δ^9-THC in dog static ataxia tests. Thus, to date, available data on the effect of chemical modification of the phenolic hydroxyl point to the free phenolic hydroxyl as the active species. It is for this reason that many investigators have accepted the tenet that a free phenolic hydroxyl is an absolute requirement for pharmacological activity.

Binder and Franke (8) accepted the idea that a free phenolic hydroxyl group was a requirement for activity and hypothesized that the hydrogen of the phenolic hydroxyl of Δ^9-THC could be involved in a hydrogen bonding interaction with a putative cannabinoid receptor. Their hypothesis is plausible, because a phenolic hydroxyl is certainly capable of donating a hydrogen in a hydrogen bonding interaction (9). It should be emphasized, however, that the oxygen of the phenolic hydroxyl at C1 not only possesses a "free" hydrogen but also possesses lone pairs of electrons, which could be involved in the recognition and activation phases of interaction with a receptor. One of these lone pairs could serve as a hydrogen acceptor in a hydrogen bonding interaction at the site of action. Such an interaction has been proposed in other drug systems. For example, the concept of proton accepting by an ether oxygen as part of a drug-receptor interaction has recently been proposed for the binding of the β-carboline, ZK 91296 to the benzodiazepine receptor (10).

Our theoretical studies of Δ^9-THC have tested the working hypothesis that there are two crucial aspects of the structure of Δ^9-THC that confer pharmacological activity upon the molecule. These components are the orientation of the lone pairs of electrons of the phenolic hydroxyl oxygen and the orientation of the C9 substituent of the carbocyclic ring (ring A; Fig. 1) relative to this oxygen (11, 12). Quantum mechanical and molecular mechanical studies of Δ^9-THC have revealed that there are two minimum energy conformations (phenol conformations I and II), representing two possible positions of the phenolic hydroxyl in Δ^9-THC (11, 12). In phenol conformation I, the phenolic hydrogen points away from the carbocyclic ring (see Fig. 2a); consequently, the lone pairs of electrons point toward this ring. The C2-C1-O-H angle is 7° (12). In phenol conformation II, the phenolic hydrogen points toward the carbocyclic ring (see Fig. 2b); consequently, the lone pairs of electrons point away from the carbocyclic ring. The C2-C1-O-H angle is 167° (12).

Simulations of a hydrogen bonding interaction between the phenolic hydroxyl oxygen of Δ^9-THC and a hypothetical hydrogen donor, using a "fairly linear" hydrogen bonding geometry (13), revealed that the phenolic hydroxyl oxygen in both phenol conformation I and II of Δ^9-THC is capable of acting as a hydrogen bond acceptor without steric interference. In phenol conformation I, this interaction was possible from the bottom face of the molecule (i.e., below the plane of the paper in Fig. 1), whereas in phenol conformation II this interaction was possible from either face (i.e., above or below the plane of the paper in Fig. 1).[1] A conformational study of the etherified cannabinoid *O*-methyl-Δ^9-THC (Δ^9-THC methyl ether, an inactive or weakly active analog) suggested that phenol conformation II of Δ^9-THC (Fig. 2b) might be the relevant conformer at its site of action, because the inactive *O*-methyl-Δ^9-THC could not mimic this conformation (14).

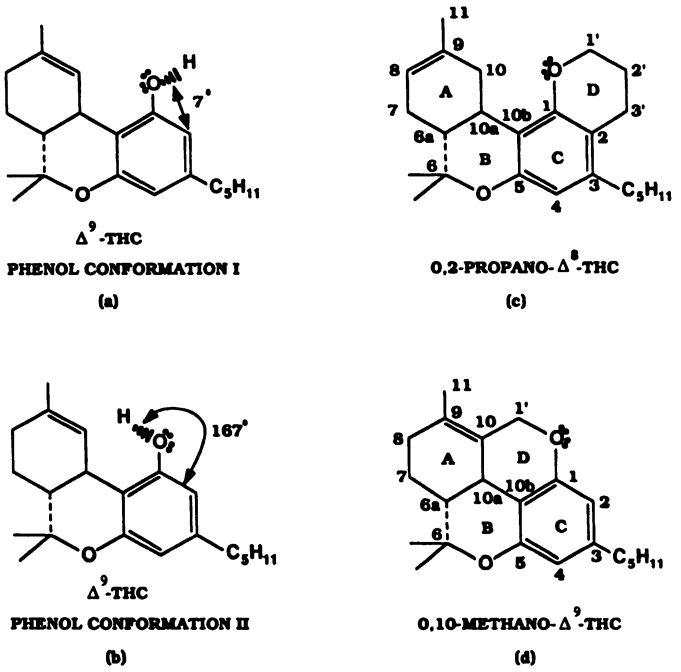


Fig. 2. a, Phenol conformation I of Δ^9-THC. The C2-C1-O-H torsion angle, which has a value of 7° [as determined by MMP2(85) (12)], is indicated by the *double-headed arrow*. b, Phenol conformation II of Δ^9-THC. The C2-C1-O-H torsion angle, which has a value of 167° [as determined by MMP2(85) (12)], is indicated by the *double-headed arrow*. c, *O*,2-Propano-Δ^8-THC. d, *O*,10-Methano-Δ^9-THC. In each of these drawings, *two pairs of dots* (according to the Lewis Dot formalism) have been drawn for each phenolic hydroxyl oxygen, in order to symbolize in two dimensions the lone pairs of electrons of this oxygen.

A study of the importance of the orientation of the C9 substituent (12) revealed that, for cannabinoids that differ only in the presence or position of a double bond in the carbocyclic ring (ring A; see Fig. 1), those that possess a C9 substituent that protrudes into the top face (above the plane of the paper in Fig. 1) of the molecule are active, whereas those whose C9 substituent lies in the plane of the aromatic ring or protrudes into the bottom face (below the plane of the paper in Fig. 1) lose activity. This finding was interpreted as evidence for a steric requirement at the site of action of these cannabinoids (i.e., that there is a region near the top of ring A that must not be blocked).

The present work represents an effort to further study whether lone pair orientation might be important for activity.

[1] P. H. Reggio, unpublished results.

We have designed two new etherified or oxygen-bridge analogs (*O*,2-propano-Δ^8-THC and *O*,10-methano-Δ^9-THC) in which the phenolic hydroxyl oxygen is incorporated into a fourth ring, such that the orientation of the lone pairs is restricted to one of two general directions (See Fig. 2, c and d). In *O*,2-propano-Δ^8-THC, the lone pairs point towards the carbocyclic ring, mimicking phenol conformation I of Δ^9-THC (Fig. 2a). In *O*,10-methano-Δ^9-THC, the lone pairs point away from the carbocyclic ring, mimicking phenol conformation II of Δ^9-THC (Fig. 2b). We have employed the idea that molecules that interact at the same site generate MEP patterns that are very similar. This MEP may be thought of as a recognition pattern for the site of action. The calculation of the MEP allows the characterization of the shape and localization of attractive and repulsive zones of varying strength in molecules. The spatial distribution and relative strength of these zones form a characteristic pattern, or "fingerprint," for a molecule in a given conformation (15).

In this paper, we report on the accessible minimum energy conformers and MEP maps generated by *O*,2-propano-Δ^8-THC and *O*,10-methano-Δ^9-THC, on a comparison of these with the conformations and MEP maps generated by Δ^9-THC, on the pharmacological evaluation of *O*,2-propano-Δ^8-THC and *O*,10-methano-Δ^9-THC, and on the implications these pharmacological results have for the cannabinoid SAR.

Materials and Methods

Theoretical studies. The crystal structure of Δ^9-THC acid B (16) was used as the starting geometry for both cannabinoids. The Modify facility within the CHEM-X (Chemical Design, Ltd., Oxford, England) molecular modeling system was used to delete unnecessary atoms and to add necessary ones at standard bond lengths and bond angles (17). The side chain in *O*,2-propano-Δ^8-THC (Fig. 2c) was shortened from pentyl to methyl and in *O*,10-methano-Δ^9-THC (Fig. 2d) from pentyl to propyl, in order to keep each molecule small enough for *ab initio* quantum mechanical calculations in a later stage of our studies. Such a modification is justifiable because the focus of this study is on the fused ring structure of these molecules and not on their side chains.

The structure of each molecule was optimized by using the method of molecular mechanics, as encoded in the MMP2(85) program.[2] Lone pairs of electrons were included in each optimization for both ether oxygens in each molecule. In order to ascertain whether any other minimum energy conformations of the fused ring structure existed, we performed MMP2(85) dihedral driver studies (18). We considered all minimum energy conformers to be accessible if they were within 3 kcal/mol of the global minimum. In the creation of our pharmacophore for cannabinoid activity, we have assumed that relevant conformations are those that statistically predominate at ordinary temperatures. The 3 kcal/mol cut-off seems reasonable, because minimum energy conformers with energies greater than 3 kcal/mol above the global minimum have a very low statistical probability of occurrence at ordinary temperatures (e.g., <1% at 298°K).

For *O*,2-propano-Δ^8-THC, the dihedral driver study of ring D required that two dihedral angles be driven. The C2-C1-O-C1′ angle (ring D) was driven from −17° to 33° in increments of 2°. The C1-C2-C3′-C2′ angle (ring D) was then driven from −45° to 19° in increments of 2–3°, while the C2-C1-O-C1′ angle was held fixed at a value of 33°. A second minimum appeared. This structure was optimized again to give the final structure. A dihedral driver study of the pyran ring (ring B) was also performed on both minimum energy ring D conformers of *O*,2-propano-Δ^8-THC. The C10b-C5-O-C6 angle (ring B) was driven from 22° to −57° in increments of 1–5°. For *O*,10-methano-Δ^9-THC, the torsion angle C10b-C1-O-C1′ in ring D was driven from 40° to −20° in increments of 2–5°. In a separate study, the torsion angle C10b-C5-O-C6 in the pyran ring (ring B) was driven from 14° to −52° in increments of 2°.

Wave functions and MEP maps were calculated for the two lowest energy conformers of each cannabinoid. The wave function of each conformer was calculated using the Gaussian 80 system of programs[3] and the LP-3G basis set (19). This basis set was designed to be used in the CHFEP scheme developed by Topiol and Osman (19). The CHFEPs are used to replace the explicit consideration of core orbitals in molecular orbital calculations. Various electronic properties, such as dipole moments and charge densities, are well reproduced in CHFEP calculations at the level of the minimal basis set LP-3G (20). The CHFEP method makes it possible to calculate wave functions for molecules as large as *O*,2-propano-Δ^8-THC and *O*,10-methano-Δ^9-THC.

The MEP was calculated using POLYPOT[4] and the resultant LP-3G wave functions. The calculated MEP is dependent on the quality of wave functions and, hence, on the quality of the basis set (21). Topiol and Weinstein[5] have shown that the MEP is well represented by wave functions obtained with an LP-3G basis set. MEP maps were obtained in planes 1.5 Å above and below the aromatic ring in each compound. The 1.5-Å distance was chosen to approximate the midpoint of the separation between two interacting molecules. This distance has been shown to yield information about the nature of the interaction between a drug molecule and its model receptor (22).

Synthesis of *O*,2-propano-Δ^8-THC and *O*,10-methano-Δ^9-THC. The synthesis of *O*,2-propano-Δ^8-THC involved alkylation of the phenolic hydroxyl oxygen of Δ^8-THC with 3-bromo-1-propanol in the presence of the base 1,8-diazabicyclo[5.4.0]undec-7-ene, in 87% yield. The resulting *O*-(3-hydroxypropyl)-Δ^8-THC was cyclized with P_2O_5 to give *O*,2-propano-Δ^8-THC in 37% yield.

The synthesis of *O*,10-methano-Δ^9-THC was achieved with a sequence of reactions that involved the cyclization of a chloroformate, in a modification of the Darzens acylation of olefins. Thus, treatment of Δ^9-THC with phosgene in the presence of *N*,*N*-dimethylaniline afforded Δ^9-THC chloroformate (94%). Subsequent intramolecular cycloaddition of the chloroformyl moiety to the Δ^9-unsaturation in the presence of $AlCl_3$ afforded the corresponding β-chloroester (51%). Treatment of the β-chloroester with lithium aluminum hydride to eliminate HCl and reduce the ester moiety gave 10-hydroxymethyl-Δ^9-THC (42%). Cyclization of the latter with *p*-toluenesulfonyl chloride in pyridine afforded *O*,10-methano-Δ^9-THC (18%). Further details of the synthesis as well as of the characterization of *O*,2-propano-Δ^8-THC and *O*,10-methano-Δ^9-THC are reported elsewhere.

Drug preparation and administration. Male ICR mice (22–30 g) and Sprague-Dawley rats (250–275 g) obtained from Dominion Laboratories (Dublin, VA) were maintained on a 14/10-hr light/dark cycle and received food and water *ad libitum*. Δ^9-THC and Δ^8-THC were obtained from the National Institute on Drug Abuse. The procedure of Olson *et al.* (23) was used to prepare micellular suspensions of the drugs for injection. Cannabinoids were dissolved (by sonication) in a 1:1 mixture of ethanol and Emulphor (EL-620, a polyoxyethylated vegetable oil; GAF Corporation, Linden, NJ). Saline (0.9% NaCl) was added to this mixture to produce a 1:1:18 ratio of ethanol/Emulphor/saline (vehicle) and the solution was further diluted with vehicle to give the desired dose of drug. Drugs were administered by the intravenous and intraperitoneal routes in the mouse and rat, respectively.

Behavioral and pharmacological evaluations. The analogs were evaluated in a battery of tests in mice that has been shown to be predictive of cannabinoid effects (5, 7). Mice were acclimated to the observation room (ambient temperature, 20–24°) overnight. Before vehicle or drug administration, rectal temperature was determined on

[2] N. L. Allinger. MMP2(85), distributed by Molecular Design, Ltd., (San Leandro, CA).

[3] B. J. Fluder, H. B. Schlegel, and S. W. Topiol. GAUSSIAN 80 (IBM version), unpublished (1980).

[4] H. Weinstein, S. Srebrenik, and R. Pauncz. POLYPOT, unpublished (1973).

[5] S. Topiol and H. Weinstein, personal communication.

partially restrained mice with a thermistor probe (inserted 25 mm) and a telethermometer (Yellow Springs Instrument Co., Yellow Springs, OH). Additionally, the latency period (sec) was measured on a standard tail-flick apparatus (24). The heat lamp of the tail-flick apparatus was maintained at an intensity sufficient to produce control latencies of 2–4 sec. Mice received tail vein injections (0.1 ml/10 g) and were placed into individual photocell activity chambers 5 min later. Locomotor activity was measured for a 10-min period, in a Digiscan animal activity monitor (Omnitech Electronics, Inc., Columbus, OH), as the number of interruptions of 16 photocell beams/chamber and was expressed as a percentage of control activity. Tail-flick latency was assessed again at 20 min after the injection, and the increase in the latency period (sec) for each mouse was recorded. An automatic heat lamp cut-off time of 10 sec was used to avoid tail injury. Rectal temperature was measured again at 60 min after the injection and the difference between pre- and postinjection values (change in degrees Celsius) was calculated for each animal. Mice were evaluated for ring immobility 1.5 hr after the injection, utilizing a slight modification of the method of Pertwee (25) as described below.

The ring immobility apparatus consisted of a 5.5-cm ring attached at a height of 16 cm to a ring stand. Each mouse was placed onto a ring for 5 min. During the 5-min period, the sum of all times during which the mouse remained motionless was measured to the nearest second. This value was divided by 300 sec and multiplied by 100 to obtain a percentage of immobility rating. The criterion for immobility was the absence of all voluntary movements (including snout and whisker). The only observable movements allowed during a period of 'immobility' were gross body movements due to breathing. If a mouse escaped from the ring by jumping or falling (due to ataxia or sedation) more than five times, then the evaluation of that animal was terminated, and the immobility index was calculated based on the total time (sec) the mouse remained on the ring. Mice that did not remain on the ring at least 2.5 min before five escapes failed to meet the minimum criteria of this evaluation, and data were disregarded.

O,2-Propano-Δ^8-THC and *O*,10-methano-Δ^9-THC were also evaluated in the rat drug discrimination paradigm. This paradigm has been shown to be highly selective for cannabinoids (26–27). For drug discrimination, rats were trained to discriminate between an injection of Δ^9-THC (3 mg/kg, intraperitoneally) and one of vehicle, given 30 min before the animals were placed into operant chambers. The protocol used for the training and testing for generalization to the Δ^9-THC cue generally followed established two-lever operant procedures (26, 28–30). Briefly, the animals were trained once a day, in 10-min sessions, to respond on one of two levers at a FR10 schedule of reinforcement for a food reward. The correct lever was determined by the preceding intraperitoneal injection (stimulus). During training sessions, only the responses on the drug lever were reinforced after an injection of Δ^9-THC (3 mg/kg), whereas responses on the opposite lever were reinforced after an injection of the vehicle. Drug and vehicle training days were scheduled on a double-alternation sequence. In order to control for possible lever bias, the lever assignments were counterbalanced across the colony, such that for half the animals the left lever was paired with reward after Δ^9-THC; the other half was trained to respond on the right lever after Δ^9-THC. An animal was considered eligible for testing when it correctly identified 8 of 10 first FRs. The number of responses/sec (response rate) was used as a measure of nonspecific central nervous system depression. Test sessions differed from training sessions in that they were only 2 min long and a completed FR on either lever was reinforced. The rats were tested 30 and 90 min after the intraperitoneal injection of the vehicle or drug on both training and test days.

To evaluate potential antagonistic properties of the test compounds, animals were pretreated 10 min before administration of Δ^9-THC or vehicle. Each experiment contained a vehicle (double-injection) control, as well as, a Δ^9-THC-positive control (vehicle/THC) and a drug/vehicle combination for comparison with the drug/THC test group. All subsequent behavioral measures were performed as described for either the rat drug discrimination or mouse multiple-evaluation paradigms.

Data analysis. Depression of locomotor activity, hypothermia, and ring immobility were expressed as percentage of control activity, change in degrees Celsius, and percentage of immobility, respectively. Antinociception was calculated as a percentage of MPE, based on the increase in the tail-flick latency period (sec) for each mouse and the maximum possible test latency of 10 sec (24). The MPE values (already defined in terms of a maximum effect of 100%) were converted to probit values and the MPE_{50} was determined by unweighted least-squares linear regression analysis of the log dose versus probit plot. A theoretical maximum effect of a given drug on percentage of control activity, change in degrees Celsius, or percentage of immobility was calculated from double-reciprocal analysis (1/effect versus 1/dose), as described by Tallarida and Murray (31). The fractional response for each dose of drug was calculated (based upon a maximum effect of 1.0 for each individual behavioral measure) and converted to probit values, and the ED_{50} was determined by unweighted least-squares linear regression analysis of the log dose versus probit plot.

Statistical analysis of dose-response data was performed using analysis of variance, with Dunnett's *t* test for comparisons with the vehicle control or Schefe's *F* test for multiple comparisons (as with antagonistic evaluations), and differences were considered significant at the $p < 0.05$ level (two-tailed). Confidence limits for the ED_{50} values were determined by the method of Litchfield and Wilcoxon (32).

Results

Theoretical Studies

Conformational analysis. Molecular mechanics studies of *O*,2-propano-Δ^8-THC revealed that two minimum energy conformers exist. Conformer II was found to be 0.81 kcal/mol higher in steric energy than conformer I, the global minimum energy structure. If we assume that there are no significant entropic or solvation differences between conformers I and II of *O*,2-propano-Δ^8-THC, the Boltzmann distribution at 298°K would predict the relative amounts of conformers I and II to be 80% and 20%, respectively. In both conformers, the cyclohexene ring exists in a half-chair conformation. The values obtained (not shown) for the torsion angles in the cyclohexene ring (ring A; Fig. 2c) of *O*,2-propano-Δ^8-THC were very similar to those obtained previously for Δ^8-THC (12).

The major difference between the two conformers of *O*,2-propano-Δ^8-THC was in the conformation of the new fourth ring (ring D; Fig. 2c). For the global minimum energy conformer, conformer I, the chroman ether 1′-CH_2 carbon is puckered into the face below the plane of the paper in Fig. 2c. The C2-C1-O-C1′ torsion angle was found to be 18°. In conformer II, this carbon is puckered into the face above the plane of the paper in Fig. 2c. The C2-C1-O-C1′ torsion angle was −17°. As determined by MMP2(85), the corresponding torsion angle in phenol conformation I of Δ^9-THC, C2-C1-O-H, was 7° (12).

In conformers I and II of *O*,2-propano-Δ^8-THC, the pyran ring (ring B; Fig. 2c) assumes a conformation such that the axial C6 methyl group is on the same side of the molecule as the H10a hydrogen and is much closer to H10a than is the other C6 methyl group. The substituents on C6 and C6a are staggered with respect to one another. The optimized C10a-C6a-C6-O values were 62° for both conformers. Dihedral driver studies revealed that a second minimum energy pyran ring conformation is possible for both ring D conformers of *O*,2-propano-Δ^8-THC. This second pyran ring conformation was similar to that described below for conformer III of *O*,10-methano-Δ^9-THC. However, this second minimum energy

pyran ring conformation was not considered accessible for either of the ring D conformations of *O*,2-propano-Δ^8-THC, because in each case it is 6.60 kcal/mol higher in steric energy than the global minimum energy conformer.

Side views of the two accessible conformers of *O*,2-propano-Δ^8-THC (conformers I and II) are presented in Fig. 3. Here the perspective is such that the new fourth ring (ring D) is closer to the viewer and the carbocyclic ring (ring A) is farther away.

The conformational analysis of the fused ring structure of *O*,10-methano-Δ^9-THC revealed that three accessible minimum energy conformers exist. Two lower energy conformers (conformers I and II) differed in steric energy by only 0.40 kcal/mol, whereas the third conformer (conformer III) was 1.99 kcal/mol higher in steric energy than the global minimum conformer (conformer I). If we assume that there are no significant entropic or solvation differences between conformers I, II, and III of *O*,10-methano-Δ^9-THC, the Boltzmann distribution at 298°K would predict the relative amounts of conformers I, II, and III to be 65, 33, and 2%, respectively. In conformer I, the cyclohexene ring was found to exist in a half-chair conformation similar to that calculated previously for Δ^9-THC (12). In conformers II and III, the cyclohexene ring exists in a distorted half-chair conformation. In conformer I, the new ether ring (ring D; Fig. 2d) puckers such that the methylene carbon (C1′) protrudes into the face above the plane of the paper in Fig. 2d. The C2-C1-O-C1′ dihedral angle was found to be −138°. For conformer II, the methylene carbon (C1′) of the new fourth ring protrudes into the face below the plane of the paper in Fig. 2d. The C2-C1-O-C1′ torsion angle was 168°. The position of the methylene carbon (C1′) in conformer III was very similar to that of conformer II, with the C1′ carbon pointing into the face below the plane of the paper in Fig. 2d. The C2-C1-O-C1′ torsion angle was 162°. Previous MMP2(85) calculations have revealed that the analogous torsion angle in Δ^9-THC, C2-C1-O-H, has a value of 167° (12).

The pyran ring (ring B; Fig. 2d) in conformers I and II of *O*,10-methano-Δ^9-THC exists in the same conformation as described above for the pyran ring in the accessible conformers of *O*,2-propano-Δ^8-THC. The C10a-C6a-C6-O torsion angle was 63° for conformer I of *O*,10-methano-Δ^9-THC and 59° for conformer II of *O*,10-methano-Δ^9-THC. In conformer III, ring B exists in a conformation such that the C6 methyl group, which is above the plane of the paper in Fig. 2d, nearly eclipses H6a along the C6-C6a bond. The C10a-C6a-C6-O torsion angle was 26° for this conformer. Fig. 4 shows side views of each accessible minimum energy conformer of *O*,10-methano-Δ^9-THC. Here the perspective is such that the new fourth ring (ring D) is closer to the viewer and the carbocyclic ring (ring A) is farther away.

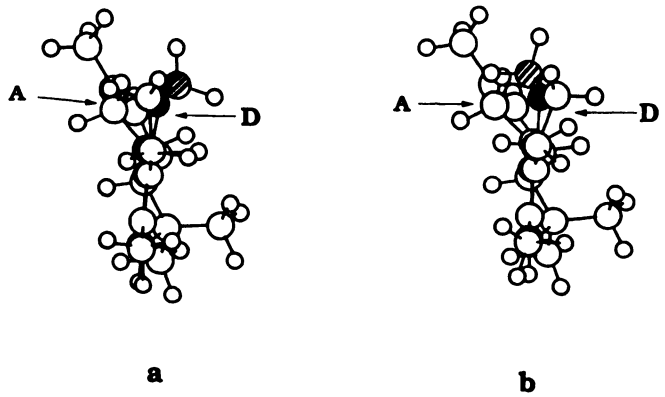


Fig. 3. Side views of conformer I (a) and conformer II (b) of *O*,2-propano-Δ^8-THC (with methyl side chain). Here the perspective of ring A is viewed in the direction of the vector from C2 to C10b. The side chain, therefore, is forward and the carbocyclic ring (ring A) (*A*) is in the back. The ring D (*D*) oxygen is shown as a *solid circle*, and the C1′ carbon is shown as a *hatched circle*.

In previous work (12), it was reported that active cannabinoids possess C9 substituents (ring A; Fig. 1) that protrude into the face of the molecule above the plane of the paper in Fig. 1. This protrusion was measured by the nonbonded torsion angle (C11-C9- -C1-O). The C11-C9- -C1-O angle was found to be −49° for Δ^9-THC and −38° for Δ^8-THC. Similarly, in all other pharmacologically active cannabinoids studied, the C11-C9- -C1-O was found to be negative (12). For all conformers of *O*,2-propano-Δ^8-THC and of *O*,10-methano-Δ^9-THC, the C11-C9- -C1-O angle was also found to be negative. For *O*,2-propano-Δ^8-THC, the C11-C9- -C1-O nonbonded torsion angle was −42° for conformer I and −41° for conformer II. For *O*,10-methano-Δ^9-THC, the C11-C9- -C1-O nonbonded torsion angle was −39° for conformer I, −22° for conformer II, and −20° for conformer III. Because *O*,2-propano-Δ^8-THC and *O*,10-methano-Δ^9-THC differ from the cannabinoids studied previously (12) by the introduction of a new fourth ring, we can conclude from their C11-C9- -C1-O angles only that any possible inactivity of these compounds cannot be attributed to the orientation of the C9 substituent of the carbocyclic ring (ring A). Here, all accessible minimum energy conformers of both *O*,2-propano-Δ^8-THC and *O*,10-methano-Δ^9-THC possess C9 substituents that protrude into the top face of the molecule, as did the C9 substituents of all active cannabinoids previously studied (12).

MEP. Both *O*,2-propano-Δ^8-THC and *O*,10-methano-Δ^9-THC are capable of generating MEPs that resemble the MEPs generated by Δ^9-THC (11). The MEP maps were calculated in planes parallel to the plane of the aromatic ring in each compound, at distances of 1.5 Å above and below the aromatic plane. The results of MEP calculations, at 1.5 Å below the plane of the aromatic ring (i.e., below the plane of the paper in Fig. 2c), for the two minimum energy conformers of *O*,2-propano-Δ^8-THC are illustrated in Fig. 5 (b and c). Also included in this figure is our previous result for the phenol conformation I of Δ^9-THC (11), which is given for comparison (Fig. 5a). In each MEP map (Fig. 5), there is a large region of negative potential, which extends from the phenolic hydroxyl oxygen (Fig. 5a) or the ring D ether oxygen (Fig. 5, b and c), over the aromatic ring, and to the pyran ring oxygen. The values of the potential in this region are very similar in all three maps illustrated in Fig. 5. For *O*,2-propano-Δ^8-THC, the MEP maps for both conformers I and II showed minima associated with the ether oxygen of ring D (Fig. 5, b and c) and with the aromatic ring (Fig. 5, b and c). Similar minima were found previously in the MEP associated with the phenolic hydroxyl oxygen (Fig. 5a) and with the aromatic ring (Fig. 5a) of Δ^9-THC in its phenol conformation I (11). MEPs at 1.5 Å above the aromatic ring for both conformers (not illustrated here) contain a large region of negative potential, which extends from the ring D ether oxygen, over the aromatic ring, and to the pyran ring oxygen. These MEPs showed minima associated with the aromatic ring and with the oxygen of the pyran ring, as does the MEP of Δ^9-THC in this plane (11). The similarity

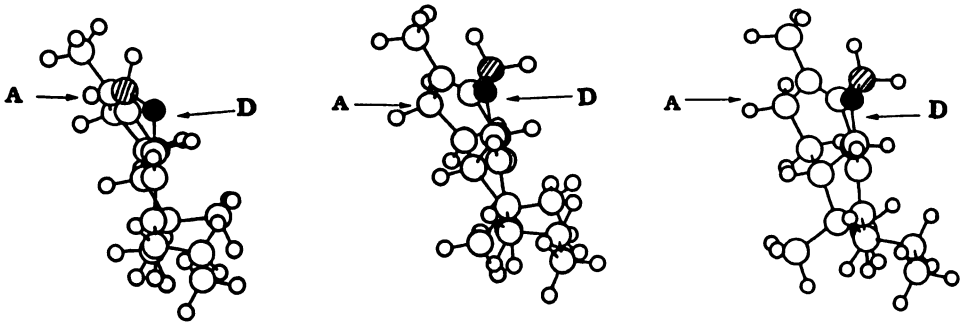


Fig. 4. Side views of conformer I (a), conformer II (b), and conformer III (c) of *O*,10-methano-Δ⁹-THC (with propyl side chain). See Fig. 3 for other details.

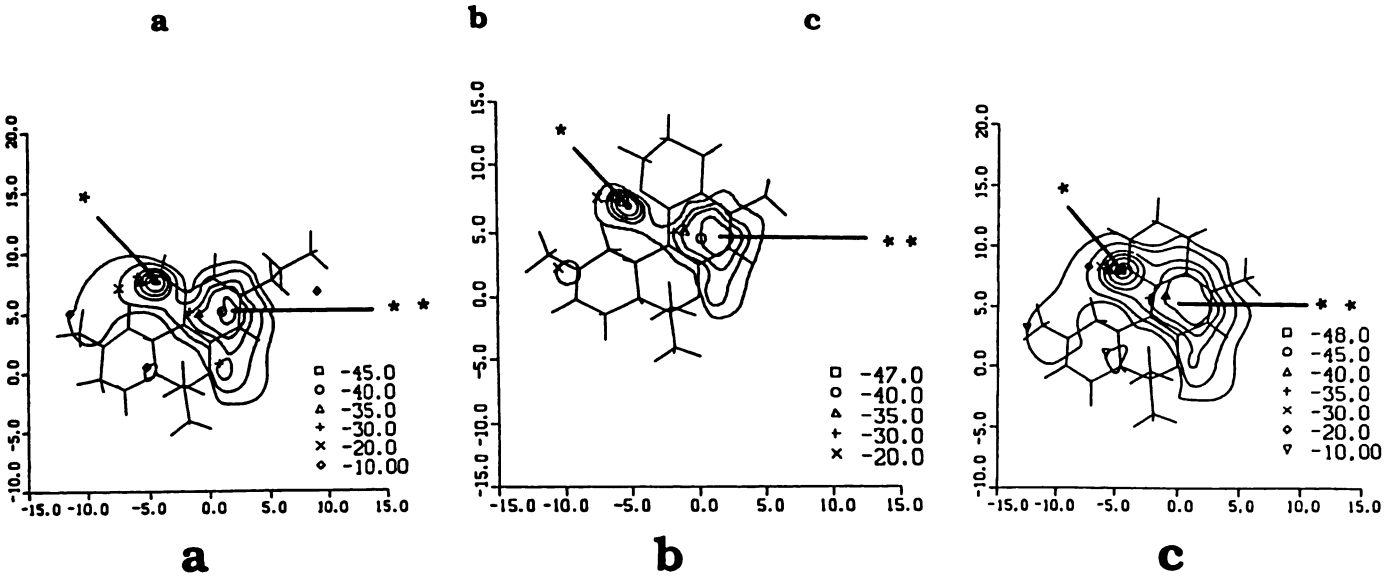


Fig. 5. Comparison of the MEPs in a plane parallel to the plane of the aromatic ring, at a distance of 1.5 Å below the aromatic plane, in phenol conformation I of Δ⁹-THC (with propyl side chain) (see Ref. 11) (a), conformer I of *O*,2-propano-Δ⁸-THC (b), and conformer II of *O*,2-propano-Δ⁸-THC (with methyl side chains) (c). *, The minimum associated with the phenolic hydroxyl oxygen in a and with the ring D oxygen in b and in c; **, minimum associated with the aromatic ring in each. The potential values are in kcal/mol.

of the MEP patterns generated by conformers I and II of *O*,2-propano-Δ⁸-THC to that generated by Δ⁹-THC in its phenol conformation I would indicate that these two conformers should be recognized by any site that recognizes Δ⁹-THC in its phenol conformation I.

Fig. 6 contains a comparison of the MEP maps of Δ⁹-THC [phenol conformation II (11)], at 1.5 Å below the plane of the aromatic ring (i.e., below the plane of the paper in Fig. 2d), with the MEP of *O*,10-methano-Δ⁹-THC in each of its two lowest energy accessible conformations (conformers I and II). It can be seen here that the MEP maps of both conformers resemble that of Δ⁹-THC in its phenol conformation II. In each MEP map (Fig. 6), there is a large region of negative potential, which extends from the phenolic hydroxyl oxygen (Fig. 6a) or the ring D ether oxygen (Fig. 6, b and c), over the aromatic ring, and to the pyran ring oxygen. A comparison of Fig. 6, a and b, reveals that the positions of the minima in conformer I (Fig. 6b) associated with the ether oxygen of ring D, the aromatic ring, and the pyran oxygen of ring B are similar to those of phenol conformation II of Δ⁹-THC, (i.e., minima associated with the phenolic hydroxyl oxygen, the aromatic ring, and the pyran ring oxygen) (Fig. 6a). The value of the potential minimum associated with the ring D ether oxygen (Fig. 6b) was less negative than that for the phenolic hydroxyl oxygen of Δ⁹-THC (Fig. 6a) (−32 versus −40 kcal/mol). The shape and localization of minima of conformer II also resembled those of Δ⁹-THC. A major difference here was that there is no potential minimum associated with the ether oxygen of ring D. There are, however, minima associated with the aromatic ring (Fig. 6c) and with the pyran oxygen of ring B (Fig. 6c). The locations of these minima were very similar to the locations of those associated with the aromatic ring (Fig. 6a) and with the pyran oxygen of ring B (Fig. 6a) of Δ⁹-THC (11). MEP maps for conformers I and II of *O*,10-methano-Δ⁹-THC, at 1.5 Å above the plane of the aromatic ring (not illustrated), contained a large region of negative potential, which extended from the ring D ether oxygen, over the aromatic ring, and to the pyran ring oxygen. Minima in the map of conformer I were associated with the aromatic ring and with the pyran oxygen (ring B), as are the minima in the MEP of Δ⁹-THC in this plane (11). Minima in the MEP map of conformer II were associated with the ring D ether oxygen, the aromatic ring, and the pyran ring oxygen.

The absence of a potential minimum associated with the ring D ether oxygen of conformer II (Fig. 6c) and the fact that the potential is much less negative in this region than it is in the phenolic hydroxyl region of Δ⁹-THC (Fig. 6a) brings into question whether conformer II of *O*,10-methano-Δ⁹-THC would be

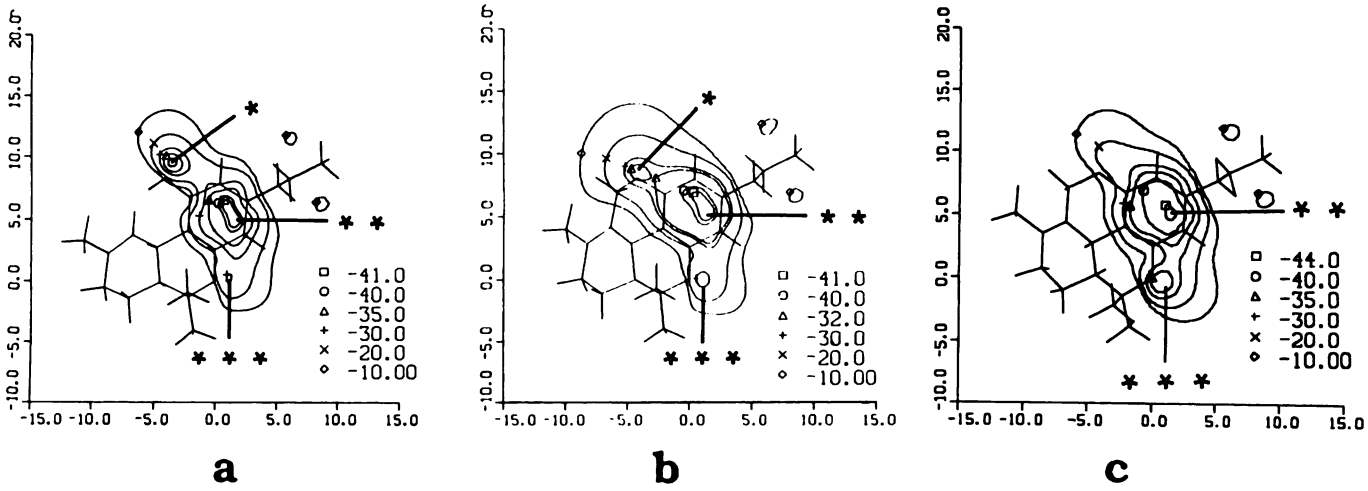


Fig. 6. Comparison of the MEPs in a plane parallel to the plane of the aromatic ring, at a distance of 1.5 Å below the aromatic plane, in phenol conformation II of Δ^9-THC with propyl side chain (see Ref. 11) (a), conformer I of *O*,10-methano-Δ^9-THC (b), and conformer II of *O*,10-methano-Δ^9-THC (with propyl side chains) (c). *, The minimum associated with the phenolic hydroxyl in a and with the ring D oxygen in b; **, the minimum associated with the aromatic ring in each; ***, the minimum associated with the pyran oxygen in each. The potential values are in kcal/mol.

recognized at a site that would recognize Δ^9-THC in its phenol conformation II. It is possible that, because the MEP map of conformer II (Fig. 6c) does possess potential values and minima similar to those of Δ^9-THC (Fig. 6a) in the aromatic ring and pyran ring oxygen regions, this conformer may still be recognized. However, the fact that conformer I does generate an MEP map (Fig. 6b) that is very similar to that generated by Δ^9-THC in its phenol conformation II permits us to conclude that *O*,10-methano-Δ^9-THC (in at least one of its forms) should be recognized by any site that recognizes Δ^9-THC in its phenol conformation II.

Structural Characterization

As mentioned above, the details of the synthesis and characterization of *O*,2-propano-Δ^8-THC and of *O*,10-methano-Δ^9-THC are reported elsewhere.[6] Of special interest was the ^{1}H NMR spectrum of *O*,10-methano-Δ^9-THC, which with the ^{13}C NMR and heteronuclear correlation spectra identified the two doublets at δ 4.52 and 4.90 as the protons of the *O*,10-methylene bridge. The upfield doublet was broadened by further splitting, which must be of a long range nature such as a "W" relationship of protons. This is in agreement with the molecular mechanics calculations reported here, which indicate that, in one of the accessible conformations of *O*,10-methano-Δ^9-THC (conformer II), the methylene is puckered into the face below the plane of the paper in Fig. 2d, such that the β-proton is in a W relationship with the 10a proton.

Behavioral and Pharmacological Evaluation

Δ^9-THC produced effects in the mouse with an average ED_{50} varying between 1.0 and 1.5 mg/kg (Table 1). Δ^8-THC proved to be as potent as Δ^9-THC in depressing locomotor activity and producing antinociception. However, Δ^8-THC was approximately 3 times less potent than Δ^9-THC in producing immobility and 10 times less potent in lowering rectal temperature. *O*,2-Propano-Δ^8-THC was effective in all of the behavioral assays, but its profile of action differed from those of both Δ^8-THC and Δ^9-THC. It was equipotent to Δ^8-THC in reducing rectal temperature and was approximately one half as potent in producing antinociception. *O*,2-Propano-Δ^8-THC was somewhat more potent than Δ^8-THC in producing immobility. This analog also did not produce a robust suppression of locomotor activity. Its ED_{50} was 5 times greater than those of Δ^8-THC and Δ^9-THC. Equally important was the fact that it did not produce depression of locomotor activity greater than 54%, whereas Δ^8-THC and Δ^9-THC produced almost 80% depression of locomotor activity. *O*,10-Methano-Δ^9-THC was completely inactive in the mouse behavioral tests at doses up to 30 mg/kg.

Analogs were also evaluated for their ability to substitute for the Δ^9-THC discriminative stimulus (Δ^9-THC cue) in the rat drug discrimination paradigm. The ED_{50} for generalization from Δ^9-THC itself was 0.6 mg/kg (Table 1). Δ^8-THC completely substituted for the Δ^9-THC cue, albeit with a 2-fold greater ED_{50}. At higher doses, these two cannabinoids result in response rate decreases (disruption) in operant experiments, with the ED_{50} values for this effect calculated to be 10.2 and 9.7 mg/kg for Δ^9- and Δ^8-THC, respectively. These results are in good agreement with published reports of Δ^9-THC as a discriminative stimulus (27) and serve to validate the efficacy of the colony.

Neither analog substituted for the Δ^9-THC cue. *O*,10-Methano-Δ^9-THC produced a modest level of drug lever responses in the dose range tested (0.3–10 mg/kg); however, limited drug availability precluded the testing of higher doses. Because the response rates had not been significantly decreased at 10 mg/kg, the possibility that this compound may generalize from the Δ^9-THC cue at higher doses cannot be ruled out. *O*,2-Propano-Δ^8-THC, on the other hand, was tested at up to 20 mg/kg with unequivocal vehicle lever selections by the rats and no response rate decreases ($p < 0.05$). At 30 mg/kg, however, severe disruption was seen, with only one of eight rats responding and with its lever presses being on the vehicle lever.

In the mouse multiple evaluation procedure, 30 mg/kg *O*,10-methano-Δ^9-THC failed to alter the pharmacological effects of 3 mg/kg Δ^9-THC. Because complete generalization was not seen with either drug, they were both tested as possible antagonists of the Δ^9-THC cue. *O*,10-Methano-Δ^9-THC at 3 mg/kg, administered 10 min before the standard training dose of Δ^9-THC (3 mg/kg), failed to alter the perception of the Δ^9-THC stimulus and consequent drug lever selection. *O*,2-Propano-Δ^8-

TABLE 1

Pharmacological activity of THC and O-bridged analogs

Pharmacological activity of THC and *O*-bridged analogs on mouse locomotion (5–15 min), tail flick antinociception (20 min), rectal temperature (60 min), and ring immobility (90–95 min) following tail vein injection, as well as activity in the rat discriminative stimulus paradigm following intraperitoneal injection. ED_{50} values are presented with their 95% confidence limits. The maximum effect produced in each mouse evaluation is indicated in parenthesis below the ED_{50} (percentage of inhibition versus control, degrees Celsius change, or percentage).

Analog	ED_{50} (95% confidence limit)				
	Motor activity	Hypothermia	Immobility	MPE	Discriminative stimulus
			mg/kg		
Δ^9-THC	1.0 (0.5–1.4) (78%)	1.4 (1.2–3.8) (−4.2°)	1.5 (0.4–2.7) (49%)	1.4 (0.5–3.4) (100%)	0.8 (0.4–1.5)
Δ^8-THC	1.9 (1.2–2.2) (79%)	15.5 (8.1–17) (−5.9°)	5.2 (3.1–8.9) (58%)	1.5 (1.0–2.3) (100%)	0.9 (0.5–1.8)
O,2-Propano-Δ^8-THC	5.4 (4.1–7.0) (54%)	16.5 (11–26) (−4.9°)	2.6 (0.3–23) (44%)	3.5 (1.6–7.2) (100%)	>30
O,10-Methano-Δ^9-THC	>30	>30	>30	>30	>10

THC at 10 mg/kg also failed to block the expected drug lever selection rate caused by administration of 1 mg/kg Δ^9-THC.

Discussion

O,10-Methano-Δ^9-THC is essentially a rigid analog, although molecular mechanics calculations reveal that three different minimum energy conformations are possible. These conformations, however, do not drastically alter the orientation of the lone pairs of electrons of the ring D ether oxygen. As can be seen in Fig. 6, both conformers I and II of *O*,10-methano-Δ^9-THC generate MEPs that are similar to the MEP generated by Δ^9-THC in its phenol conformation II. Thus, this analog resembles the phenol conformation II attained by Δ^9-THC, where the lone pairs project away from ring A (see Fig. 2b). *O*,10-Methano-Δ^9-THC appeared to be non-THC-like in the multiple evaluation procedure in mice, as well as in the rat discriminative stimulus model, at the doses tested. Higher doses could not be administered in these paradigms due to limited solubility. Additionally, this analog failed to modify or antagonize the pharmacological effects of Δ^9-THC.

There are several possible explanations for the lack of pharmacological activity of *O*,10-methano-Δ^9-THC. The inactivity might be attributed to inappropriate orientation of the lone pairs, although it is equally possible to argue that the lack of activity is due to the lack of a free phenolic hydroxyl group. A corollary to the latter idea is that pharmacological activity is conferred by Δ^9-THC acting as the hydrogen donor in hydrogen bonding and not by it acting as a hydrogen acceptor via a lone pair of electrons, the latter being one of the basic hypotheses evaluated here. However, results discussed below indicate pharmacological activity is observed in the absence of a free phenolic hydroxyl when the lone pairs are oriented in essentially the opposite direction. Another possible reason for the inactivity of *O*,10-methano-Δ^9-THC might be that the C1′ carbon of the ring D methylene sterically prevents interaction with the site of action. This reasoning suggests that Δ^9-THC is acting specifically at some molecular site, such as a receptor. This steric hindrance could conceivably prevent a pharmacological action regardless of whether a free phenolic hydroxyl exists or lone pairs are properly oriented. However, the possibility of steric hindrance by the C1′ carbon of ring D seems less likely, due to the fact that 10-methylene-Δ^8-THC is a very potent cannabimimetic.[6] The methylene group of this analog projects into nearly the same region of space filled by the C1′ carbon of *O*,10-methano-Δ^9-THC (conformer I). The failure of *O*,10-methano-Δ^9-THC to produce any pharmacological effect, then, suggests that its lone pair orientation (away from the carbocyclic ring) is not a lone pair orientation recognized at the site(s) of action of Δ^9-THC.

O,2-Propano-Δ^8-THC is also essentially a rigid analog, with two slightly different accessible conformations revealed by molecular mechanics calculations. However, these conformations also do not drastically alter the orientation of the lone pairs of electrons of the ether oxygen in ring D. Both conformers I and II of *O*,2-propano-Δ^8-THC generate MEPs that are very similar to that generated by Δ^9-THC in its phenol conformation I (Fig. 5). Thus, this analog resembles the phenol conformation I attained by Δ^9-THC, where the lone pairs project toward ring A (see Fig. 2a). This analog, in contrast to *O*,10-methano-Δ^9-THC, does possess pharmacologically relevant characteristics. The potency of *O*,2-propano-Δ^8-THC varies from 0.2- to 2.0-fold that of Δ^8-THC. The full spectrum of effects observed with this analog in the mouse suggests that it should be considered a cannabimimetic drug. However, there are two important aspects to the pharmacology of this analog. Unlike either Δ^8-THC or Δ^9-THC, this analog produces only moderate suppression of mouse locomotor activity. The efficacy of *O*,2-propano-Δ^8-THC in reducing motor activity is almost half of that observed for Δ^8-THC and Δ^9-THC. This efficacy suggests that *O*,2-propano-Δ^8-THC is partially devoid of central nervous system sedative properties. The other peculiar aspect of the pharmacology of *O*,2-propano-Δ^8-THC is its inability to substitute for Δ^9-THC in the rat discriminative stimulus model. Because this model has long been considered predictive of those compounds capable of producing cannabinoid effects, the results obtained with *O*,2-propano-Δ^8-THC could be interpreted as an indication that this analog is not THC-like. On the other hand, this inability could be interpreted as an indication that a single mechanism is not responsible for the production of all cannabimimetic responses. However, a wealth of possible explanations exist for the lack of generalization to *O*,2-propano-Δ^8-THC. For example, because the metabolism and disposition of *O*,2-propano-Δ^8-THC have not been studied, we cannot rule out the possibility that differential solubility or pharmacoki-

[6] H. H. Seltzman, D. R. Compton, and B. R. Martin, unpublished observations.

netic differences between Δ^9-THC and *O*,2-propano-Δ^8-THC could contribute to the failure of *O*,2-propano-Δ^8-THC to generalize in the rat discriminative stimulus model.

The commonly accepted interpretation of cannabinoid SAR, that all of the pharmacological activities of Δ^9-THC require the free phenolic hydroxyl, presumably acting as a hydrogen donor in a hydrogen bonding interaction, is clearly not tenable. Results obtained with the etherified cannabinoid *O*,2-propano-Δ^8-THC indicate that the basic hypothesis being evaluated here, which is that the orientation of the phenolic hydroxyl oxygen lone pair electrons (possibly acting as a hydrogen acceptor in a hydrogen bonding interaction) may be important to cannabinoid activity, seems to be true for at least some of the pharmacological effects. The cannabinoid activity of *O*,2-propano-Δ^8-THC implies that lone pair orientation toward ring A of Δ^9-THC may be sufficient for producing most THC-like effects.

The conformation attained by Δ^9-THC methyl ether (in terms of lone pair orientation) is nearly identical to that observed for *O*,2-propano-Δ^8-THC (14). However, unlike that observed with *O*,2-propano-Δ^8-THC, Δ^9-THC methyl ether and Δ^8-THC methyl ether analogs have been considered to be essentially inactive cannabinoids. Based on the reported inactivity of these cannabinoids in a rhesus monkey behavioral assay (6), the conclusion was drawn in a previous study that phenol conformation II of Δ^9-THC, rather than phenol conformation I, might be important for activity (14). The results obtained here, however, indicate just the opposite. These new results imply that the orientation of the lone pairs of electrons toward the carbocyclic ring (phenol conformation I) may be important for some of the pharmacological effects of the cannabinoids and that the alternate orientation (phenol conformation II) is probably not important for any of the pharmacological effects of the cannabinoids. Based on data presented here, it is unclear why Δ^9-THC methyl ether and Δ^8-THC methyl ether should be inactive. There is reason to investigate the possible agonist/antagonist properties of these drugs more closely and to more thoroughly determine the exact potencies of these analogs in a wider variety of behavioral evaluations than has previously been published.

Acknowledgments

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References

1. Mechoulam, R., and Y. Gaoni. Recent advances in the chemistry of hashish. *Fortschr. Chem. Org. Naturst.* **25**:175–213 (1967).
2. Gaoni, Y., and R. Mechoulam. Isolation, structure, and partial synthesis of an active constituent of hashish. *J. Am. Chem. Soc.* **86**:1646–1647 (1964).
3. Gaoni, Y., and R. Mechoulam. The isolation and structure of *delta*-1-tetrahydrocannabinol and other neutral cannabinoids from hashish. *J. Am. Chem. Soc.* **93**:217–224 (1971).
4. Devane, W. A., F. F. Dysarz, M. R. Johnson, S. Melvin, and A. C. Howlett. Determination and characterization of a cannabinoid receptor in rat brain. *Mol. Pharmacol.* **34**:605–613 (1988).
5. Razdan, R. K. Structure-activity relationships in cannabinoids. *Pharmacol. Rev.* **38**:75–149 (1986).
6. Edery, H., Y. Grunfeld, G. Porath, Z. Ben-Zvi, A. Shani, and R. Mechoulam. Structure-activity relationships in the tetrahydrocannabinol series. *Arzneim.-Forsch.* **22**:1995–2003 (1972).
7. Martin, B. R., L. S. Harris, and W. L. Dewey. Pharmacological activity of *delta*-9-THC metabolites and analogs of CBD, *delta*-8-THC, and *delta*-9-THC, in *The Cannabinoids: Chemical, Pharmacologic, and Therapeutic Aspects* (S. Agurell, W. L. Dewey, and R. E. Willette, eds.). Academic Press, Orlando, FL, 523–544 (1984).
8. Binder, M., and I. Franke, Is there a THC receptor?, in *Neuroreceptors* (F. Hucho, ed.). Walter de Guyter, New York, 151–161 (1982).
9. Scheiner, S. Theoretical studies of proton transfers. *Acc. Chem. Res.* **18**:174–180 (1985).
10. Villar, H. O., E. T. Uyeno, L. Toll, W. Polgar, M. F. Davies, and G. H. Loew. Molecular determinants of benzodiazepine receptor affinities and anticonvulsant activities. *Mol. Pharmacol.* **36**:589–600 (1989).
11. Reggio, P. H., and A. P. Mazurek. A molecular reactivity template for cannabinoid activity. *J. Mol. Struct.* **149**:331–343 (1987).
12. Reggio, P. H., K. V. Greer, and S. M. Cox. The importance of the orientation of the C-9 substituent to cannabinoid activity. *J. Med. Chem.* **32**:1630–1635 (1989).
13. Murray-Rust, P., and J. P. Glusker. Directional hydrogen bonding to sp^2 and sp^3 hybridized oxygen atoms and its relevance to ligand-macromolecule interactions. *J. Am. Chem. Soc.* **106**:1018–1025 (1984).
14. Reggio, P. H. Molecular determinants for cannabinoid activity: refinement of a molecular reactivity template. *Natl. Inst. Drug Abuse Res. Monogr. Ser.* **79**:82–95 (1987).
15. Politzer, P., and D. Truhlar. *Chemical Applications of Atomic and Molecular Electrostatic Potentials.* Plenum Press, New York (1981).
16. Rosenqvist, E., and T. Ottersen. The crystal and molecular structure of *delta*-9-tetrahydrocannabinolic acid B. *Acta Chem. Scand.* **1329**:379–384 (1975).
17. Mitchell, A. D., and L. C. Cross, eds. *Tables of Interatomic Distances and Configurations in Molecules and Ions*, Special Publications 11 and 18. The Chemical Society, London (1958, 1965).
18. Burkert, U., and N. L. Allinger. *Molecular Mechanics: American Chemical Society Monograph Series 177.* American Chemical Society, Washington, D. C. (1982).
19. Topiol, S., and R. Osman. On the use of minimal valence basis sets with the coreless Hartree-Fock effective potential. *J. Chem. Phys.* **73**:5191–5196 (1980).
20. Topiol, S., R. Osman, and H. Weinstein. Effective potential methods for use in electronic structure calculations of large molecules. *Ann. N. Y. Acad. Sci.* **367**:17–34 (1981).
21. Weinstein, H., R. Osman, J. P. Green, and S. Topiol. Electrostatic potentials as descriptors of molecular reactivity: the basis for some successful predictions of biological activity, in *Chemical Applications of Atomic and Molecular Electrostatic Potentials* (P. Politzer and D. G. Truhlar, eds.). Plenum Press, New York, 309–334 (1981).
22. Weinstein, H., D. Chou, S. Kang, C. L. Johnson, and J. P. Green. Reactivity characteristics of large molecules and their biological activity: indolealkylamines on the LSD/serotonin receptor. *Int. J. Quantum Chem. Quantum Biol. Symp.* **3**:135–150 (1976).
22a. Seltzman, H. H., Y.-A. Hsieh, C. G. Pitt, and P. H. Reggio. Synthesis of Rotationally Restricted Tetrahydrocannabinol Ethers. *J. Org. Chem.*, in press.
23. Olson, J. L., M. Makhani, K. H. Davis, and M. E. Wall. Preparation of Δ^9-tetrahydrocannabinol for intravenous injection. *J. Pharm. Pharmacol.* **25**:344 (1973).
24. Dewey, W. L., L. S. Harris, J. F. Howes, and J. A. Nuite. The effect of various neurohumoral modulators on the activity of morphine and the narcotic antagonists in tail-flick and phenylquinone test. *J. Pharmacol. Exp. Ther.* **175**:435–442 (1970).
25. Pertwee, R. G. The ring test: a quantitative method for assessing the "cataleptic" effect of cannabis in mice. *Br. J. Pharmacol.* **46**:753–763 (1972).
26. Weissman, A. Generalization of the discriminative stimulus properties of Δ^9-THC to cannabinoids with therapeutic potential, in *Stimulus Properties of Drugs: Ten Years of Progress* (F. C. Colpaert and J. A. Rosecrans, eds.). Elsevier, Amsterdam, North Holland, 99–122 (1978).
27. Browne, R. G., and A. Weissman. Discriminative stimulus properties of Δ^9-tetrahydrocannabinol: mechanistic studies. *J. Clin. Pharmacol.* **21**:227S–234S (1981).
28. Ford, R. D., R. L. Balster, W. L. Dewey, J. A. Rosecrans, and L. S. Harris. The discriminative stimulus properties of Δ^9-THC: generalization to some metabolites and congeners, in *The Cannabinoids: Chemical, Pharmacologic, and Therapeutic Aspects* (S. Agurell, W. L. Dewey, and R. E. Willette, eds.). Academic Press, New York, 545–561 (1984).
29. Järbe, T. U. C., and D. E. McMillan. Discriminative stimulus properties of tetrahydrocannabinols and related drugs in rats and pigeons. *Neuropharmacology* **18**:1023–1024 (1979).
30. Martin, B. R., M. J. Kallman, G. F. Kaempf, L. S. Harris, and W. L. Dewey. Pharmacological potency of *R*- and *S*-3′-hydroxy-Δ^9-tetrahydrocannabinol: additional structural requirement for cannabinoid activity. *Pharmacol. Biochem. Behav.* **21**:61–65 (1984).
31. Tallarida, R. J., and R. B. Murray, eds. *Manual of Pharmacologic Calculations with Computer Programs.* Springer-Verlag, New York (1986).
32. Litchfield, L. T., and F. A. Wilcoxon. A simplified method of evaluating dose effect experiments. *J. Pharmacol. Exp. Ther.* **96**:99–113 (1949).

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