Characterization of a Region of Steric Interference at the Cannabinoid Receptor Using the Active Analog Approach[†]

Patricia H. Reggio,* Al M. Panu, and Scott Miles

Department of Chemistry, Kennesaw State College, Marietta, Georgia 30061

Received July 29, 1992

In this paper, it is hypothesized that the distinction between certain active and inactive cannabinoids is that the inactive analogs possess extra volume associated with their carbocyclic rings that may be responsible for an unfavorable interaction at the cannabinoid receptor. Using the active analog approach, a model is developed of a region of steric interference at this receptor using the active cannabinoids (−)-*trans*-Δ^9-tetrahydrocannabinol, (−)-*trans*-Δ^8-tetrahydrocannabinol, (−)-11-hydroxy-β-hexahydrocannabinol, and a (−)-*trans*-11-hydroxy-Δ^8-tetrahydrocannabinol dimethylheptyl derivative and the inactive cannabinoids (9S,6aR)-*trans*-$\Delta^{10,10a}$-tetrahydrocannabinol and a (+)-*trans*-11-hydroxy-Δ^8-tetrahydrocannabinol dimethylheptyl derivative. Each of these molecules satisfy the cannabinoid pharmacophoric requirements, i.e., a phenolic oxygen at C1 and a side chain of acceptable length at C3. Accessible conformers of each molecule were identified by using the method of molecular mechanics as encoded in the MMP2(85) program. The MAP facility within the Chem-X molecular modeling program was then used to calculate the region of steric interference (termed the receptor essential volume, REV) from these accessible conformers. The calculations revealed an REV region located near the top of the carbocyclic ring in the bottom face of the molecule. In order to explore the use of this REV to account for the activities of other cannabinoids, the minimally active classical cannabinoid (−)-11-hydroxy-α-hexahydrocannabinol, an active benzofuran cannabinoid, and the active nonclassical cannabinoid CP-47,497 were then studied. In each case, the activity or minimal activity of each compound can be explained on the basis of the ability of one or more accessible conformer of each molecule to clear the REV calculated here. The results of this study provide an explanation at the molecular level for observed activity differences between cannabinoids that exhibit shape differences associated with their carbocyclic rings.

Introduction

The cannabinoids are the group of C_{21} compounds found naturally occurring in the Indian hemp *Cannabis sativa* L.[1] Both hashish and marijuana are derived from cannabis and have been used for centuries for their medicinal, as well as for their psychotomimetic, effects. (−)-*trans*-Δ^9-Tetrahydrocannabinol ((−)-Δ^9-THC, 1; see Chart I), has been reported to be the major psychoactive ingredient in cannabis.[2] It is generally agreed that a pharmacophore for cannabinoid activity must include the presence of a phenolic oxygen at C1 and the presence of a side chain of acceptable length (propyl or higher) at C3.[3] Yet these criteria alone cannot explain why, for example, (−)-Δ^9-THC (Figure 1a) is an active cannabinoid and (−)-Δ^7-THC (Figure 1b) is an inactive cannabinoid. Results presented here indicate that the distinction between such active and inactive cannabinoids hinges on steric discrepancies. More specifically, we suggest that the inactive analogs possess steric components that may be responsible for an *unfavorable interaction* at the cannabinoid receptor. Using the active analog approach,[4] we identify a region of steric interference that we propose to be responsible for such unfavorable steric interactions.

Over the years, various investigators have noted that there is a noticeable shape difference between certain active and inactive cannabinoid analogues.[5–7] However, no explanations have been offered based on detailed studies of these shape differences and their possible meanings for interaction at the cannabinoid receptor. In our early study

of cannabinoids that differed from (−)-Δ^9-THC only in the position or absence of unsaturation in the carbocyclic ring, we found a correlation between activity and the orientation of the C9 substituent of the carbocyclic ring.[8] Here, the nonbonded "protrusion" torsion angle (C11–C9––C1–O, τ) was used to quantitate this orientation. In general, we found that those cannabinoids in the set that possessed negative values of τ were active. A negative value of τ means that the C9 substituent points into the top or β face of the molecule or to the left of the phenyl group hydroxyl oxygen when the molecule is viewed in a sideway perspective (see Figure 1a). Furthermore, we found that those cannabinoids that possessed positive values of τ were inactive. A positive value of τ means that the C9 substituent points into the bottom or α face of the molecule or to the right of the phenyl group hydroxyl oxygen when the molecule is viewed in a sideway perspective (see Figure 1b). Our results for the correlation of τ values with activity implied that a simple structural feature, a C9 substituent protruding into the bottom face of the molecule (τ greater than zero), can abolish or significantly diminish activity. This finding has led us to hypothesize that at the cannabinoid receptor there may be a region of steric interference that must be cleared in order for a cannabinoid to fit properly at this site. With respect to the binding molecule, this critical area would appear to be located near the top of the carbocyclic ring in the bottom face of the molecule.

In this paper, we describe the first test of our hypothesis concerning a specific region of steric interference. This region is termed the receptor essential volume (REV) and is that region of space occupied by the atoms of inactive analogs that is *not occupied* by atoms of active analogs.

---

* To whom correspondence should be addressed.

Chart I

1

2

3

4

5

6

a   b

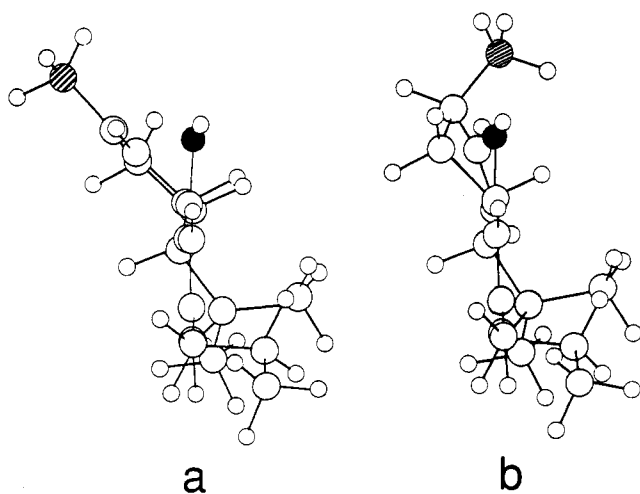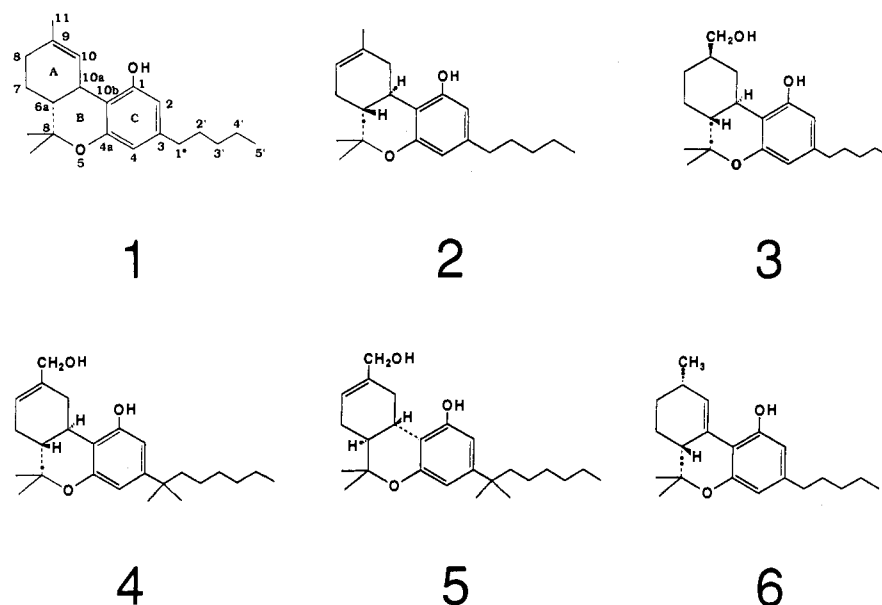**Figure 1.** Conformations of the global-minimum-energy conformer of (a) the active cannabinoid (-)-Δ^9-THC (with propyl side chain) and (b) the inactive cannabinoid (-)-Δ^7-THC (with propyl side chain) as determined by MMP2(85). The perspective of the carbocyclic ring (ring A) in each is viewed in the direction parallel to the vector from C2 to C10b. Here the oxygen of the phenyl group hydroxyl in each is shown as a blackened circle and the carbon of the C9 substituent (i.e, the C11 carbon) in each is shown as a hatched circle.
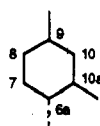
The calculation of the REV can assist in defining the location of the receptor with respect to the binding molecule, because the REV map can be interpreted to represent that region of space occupied by atoms of the receptor itself.[4] Here, we report on the conformational analysis of a series of compounds, 1–6 (see Chart I), that served in the identification of possible conformers for the calculation of the REV map. We discuss the selection of those accessible conformers of molecules 1–6 that possess the essential pharmacophoric features and were therefore included in the REV calculation. We present a cannabinoid REV map constructed according to the active analog approach using the conformers of 1–6 identified above. Finally, we explore the ability of this newly generated cannabinoid REV map to account for the activities of both classical and nonclassical cannabinoids 7–9 (see Chart II).

Chart II

7   8

9
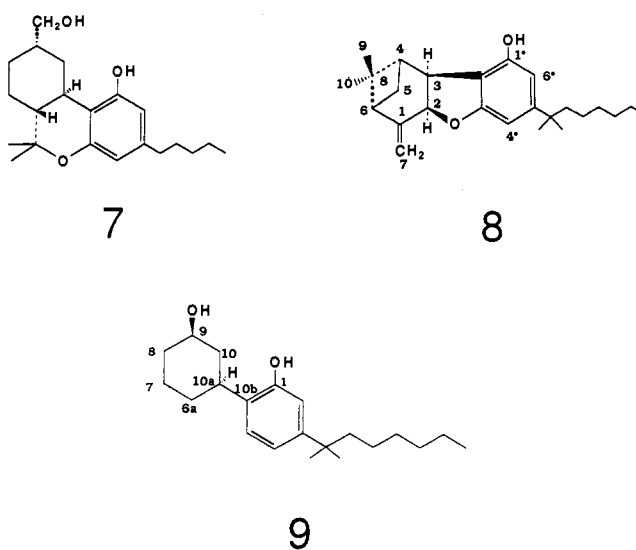
Results and Discussion

Criteria and Compounds Used in Construction of the REV. Several criteria were used in the selection of subject molecules for the construction of the cannabinoid REV map in order to permit the study of a set of cannabinoids whose major differences were in their carbocyclic rings. (1) All cannabinoids had to be structurally related to (-)-Δ^9-THC (1). Thus, the compounds must possess the same fused-ring structure as 1, but differ only in the position of unsaturation or of substitution in the carbocyclic ring or in the stereochemistry of the attachment of rings A and B (Chart I). (2) Because our work[9] and that of others[3] has led to the conclusion that the phenyl group hydroxyl at C1 is important for cannabinoid activity, all cannabinoids had to possess a free phenyl group hydroxyl at C1 whose minimum-energy orientations mimicked those of known cannabimimetics like (-)-Δ^9-THC.[9] (3) Because the C3 side chain is thought to be required for cannabinoid activity, all subject molecules chosen had to possess C3 side chains of acceptable length. Cannabinoid SAR studies have shown that the C3 side chain must be propyl or higher in order for the

Table I. Carbocyclic Ring Torsion Angles[a]

conformation	ω_1 (deg)	ω_2 (deg)	ω_3 (deg)	ω_4 (deg)	ω_5 (deg)	ω_8 (deg)
			$(-)$-Δ^9-THC			
half-chair, B1	37	−66	62	−32	4	−6
half-chair, B2	52	−61	38	−8	−1	−22
			$(-)$-Δ^8-THC			
half-chair, B1	17	−51	67	−48	15	2
			$(-)$-11-OH-β-HHC			
chair, B1	55	−61	63	−61	56	−53
twist, B1	−34	−29	68	−43	−17	57
chair, B2	58	−53	49	−52	57	−59
			$(-)$-11-OH-Δ^8-THC-DMH			
half-chair, B1	17	−51	67	−47	14	2
			$(+)$-11-OH-Δ^8-THC-DMH			
half-chair, B1	−17	51	−67	47	−14	−2
			$(9S,6aR)$-$\Delta^{10,10a}$-THC			
half-chair, B1	62	−50	20	−2	14	−44
half-chair 2, B1	−59	30	1	−2	−30	56
			$(-)$-11-OH-α-HHC			
chair, B1	54	−61	63	−60	54	−50
twist, B1	−30	−34	67	−32	−30	64
chair, B2	57	−52	48	−51	56	−57
			CP-47, 497			
eq–eq, chair	56	−56	56	−56	57	−57
eq–eq, twist	−63	31	31	−65	33	29
ax–ax, chair	−53	50	−48	52	−58	57

[a] ω_1 = C9–C8–C7–C6a, ω_2 = C8–C7–C6a–C10a, ω_3 = C7–C6a–C10a–C10, ω_4 = C6a–C10a–C10–C9, ω_5 = C10a–C10–C9–C8, ω_8 = C10–C9–C8–C7.

molecules to exhibit activity. In addition, branching of the side chain has been shown to increase potency.[3] The compounds in this study possess either straight alkyl (pentyl) or branched alkyl (dimethylheptyl) side chains at C3. (4) Because binding data for the cannabinoid receptor is very limited at present, molecules had to have been assayed in the same pharmacological test for cannabinoid activity. All molecules chosen for study had been evaluated in a drug-discrimination assay in pigeons. Such discrimination data has been reported to be reflective of or highly correlated with the subjective "marijuana high" in humans.[10] Using these criteria, we chose the active cannabinoids $(-)$-*trans*-Δ^9-tetrahydrocannabinol [$(-)$-Δ^9-THC (1)[11]], $(-)$-*trans*-Δ^8-tetrahydrocannabinol[$(-)$-Δ^8-THC(2)[12]], $(-)$-11-hydroxy-β-hexahydrocannabinol[$(-)$-11-OH-β-HHC (NL-105, 3)[13]], and a $(-)$-11-hydroxy-Δ^8-tetrahydrocannabinol dimethylheptyl derivative [$(-)$-11-OH-Δ^8-THC-DMH (4)[14]] and the inactive cannabinoids $(+)$-11-OH-Δ^8-THC-DMH (5)[14] and $(9S, 6aR)$-$\Delta^{10,10a}$-tetrahydrocannabinol [$(9s,6aR)$-$\Delta^{10,10a}$-THC (6)[15]]; see Chart I.

In order to explore the use of the cannabinoid REV map once calculated, the minimally active classical cannabinoid 11-OH-α-HHC (NL-106, 7),[13] an active benzofuran cannabinoid (8),[16] and an active nonclassical cannabinoid (CP-47,497, 9)[17,18] (see Chart II) were studied. Each of these molecules was chosen because a casual inspection of its structure might lead to an incorrect assessment of its chance for activity. Like the cannabinoids used to build the REV map, the pharmacological activity of each of these compounds has been assayed in drug-discrimination tests in pigeons.

Conformational Analysis. In order to construct an REV using the molecules in Chart I and then explore its

utility within the molecules in Chart II, it was first necessary to study all accessible energy minima of fused-ring conformers in these molecules. Conformational analyses were performed using the method of molecular mechanics as detailed in the Experimental Section. Tables I and II summarize the conformational results. Table I lists the carbocyclic ring torsion angles for each accessible conformer identified for molecues 1–7 and 9. Table II summarizes all of the *accessible* fused-ring conformers identified for molecules 1–9. In each case, the form listed in Table II is the one in which all rotatable groups (or rings) are in their lowest energy positions. Side views of the accessible conformers (without side chains) of the active cannabinoids 1–4 are depicted in Figure 2, those of the inactive cannabinoids 5 and 6 are shown in Figure 3, and those of the test case cannabinoids 7–9 are depicted in Figure 4.

REV Calculation. As detailed in the Experimental Section, the active analog approach of Marshall[4] was used to construct the REV. Figures 5 and 6 illustrate the process and the results of these calculations. The union of the volumes of accessible conformers of active molecules 1–4, termed the excluded volume map (see Figure 5a) was calculated.[4] Next, the unique volume requirements of each accessible conformer of the inactive cannabinoids were determined (Figure 5b–d). In each case, this unique volume is the excess volume which is not shared by the inactive conformer with the set of active molecules (i.e., the excluded volume map, Figure 5a). Finally, the intersection of the unique volumes of the inactive molecules was calculated (see Figure 6). This volume is termed the receptor essential volume (REV) and represents that unique volume common to all accessible conformers of the inactive molecules. The REV can be interpreted as

Table II. Accessible Conformers of Molecules 1–9[a]

conformation	ring B C10a–C6a–C6–O (deg)	ring A–ring C Cl–C10b–C10a–C10 (deg)	phenol C2–C1–O–H (deg)	CH2OH[b] C10–C9–C11–O (deg)	ΔFSE (kcal/mol)	τ C11–C9—Cl–O (deg)
			(-)-Δ9-THC			
half-chair, B1	63	-52	7	N/A	0.00	-49
half-chair, B2	29	-3	-3	N/A	3.77	-1
			(-)-Δ8-THC			
half-chair, B1	62	-54	6	N/A	0.00	-38
			(-)-11-OH-β-HHC			
chair, B1	63	-53	8	173	0.00	-53
twist, B1	61	-55	6	173	5.53	-132
chair, B2	17	-10	1	173	4.81	-10
			(-)-11-OH-Δ8-THC-DMH			
half-chair, B1	62	-54	6	-60	0.00	-39
			(+)-11-OH-Δ8-THC-DMH			
half-chair, B1	62	54	-6	60	0.00	38
			(9S,6aR)-Δ10,10a-THC			
half-chair, B1	59	1	0	N/A	0.00	68
half-chair 2, B1	61	13	-6	N/A	2.87	45
			(-)-11-OH-α-HHC			
chair, B1	63	-53	8	-170	0.00	47
twist, B1	61	-53	5	-173	1.67	-24
chair, B2	22	-13	1	-178	4.93	98
			Benzofuran			
N/A	N/A	N/A	1	N/A	0.00	N/A
			CP-47,497			
eq–eq, chair	N/A	-117	0	65	0.00	N/A
eq–eq, twist	N/A	-131	1	61	5.73	N/A
ax–ax, chair	N/A	-67	3	171	5.84	N/A

[a] Lower energy phenol and lowest energy CH2OH position where applicable. [b] For CP-47,497, the value listed is for the C10–C9–O–H torsion angle.

a region of space delineated by the site of action and therefore unavailable for occupation by atoms of the drug molecule. It, therefore, represents a region of steric interference at the site of action of these compounds.[4]

Figure 6 depicts the REV map in relation to the global-minimum conformation of (-)-Δ9-THC (1) from two different perspectives. This REV is a large region of steric interference (as we had hypothesized)[8,19] located near the top of the carbocyclic ring in the bottom face of the molecule. The calculation also revealed a very small second region of steric interference near the outer edge of the carbocyclic ring in the bottom face of the molecule. When the REV calculation was repeated at a density of 1 point per Å, this small second region disappeared, while the larger REV region near the top of the carbocyclic ring remained. The disappearance of this small region indicates that the larger REV region near the top of the carbocyclic ring represents the most significant volume difference between the active and inactive cannabinoids studied.

Use of REV To Explain Activity. The REV calculated here can now be considered an added element in the pharmacophore for cannabinoid activity. In order to explore the use of this new pharmacophoric element, it was necessary to evaluate the ability of the new model to predict the chances for activity in molecules that were not used in the REV construction. For this purpose, we chose both classical and nonclassical cannabinoid compounds.

11-OH-α-HHC (NL-106, 7). The classical cannabinoid 11-OH-α-HHC is an interesting test case molecule because, in its global-minimum-energy conformer, the C9 substituent protrudes into the bottom face ($\tau > 0$), thus mimicking the shape of inactive cannabinoids. Yet, 11-OH-α-HHC exhibits activity in all pharmacological assays including drug discrimination ($ED_{50} = 1.72$ mg/kg), albeit at a

reduced level in comparison to its more active epimer, 11-OH-β-HHC ($ED_{50} = 0.02$ mg/kg).[13,17] While other investigators have noted the shape difference between the global-minimum conformers of active and inactive cannabinoids,[5-7] no other investigators have offered a hypothesis as to how compounds such as 11-α-OH-HHC can present "inactive" shapes and still retain activity. Results obtained here (see Experimental Section) suggest that the answer to this apparent contradiction lies in considering *all* accessible conformers of 11-α-OH-HHC and not simply its global-minimum-energy conformer. As summarized in Table II and depicted in Figure 4a–c, our MMP2(85) calculations (see Experimental Section) revealed three accessible fused ring conformers of 7. In the global-minimum-energy conformer (Figure 4a), the carbocyclic ring exists in a chair conformation and the dihydropyran ring, ring B, exists in a half-chair conformation (i.e., chair, B1; Table II). In the conformer which is 1.67 kcal/mol above the global minimum (Figure 4b), the carbocyclic ring exists in a twist conformation, and the dihydropyran ring (ring B) exists in a half-chair conformation (i.e., twist, B1; Table II). The final steric energy of the chair, B2 form where the dihydropyran ring exists in a boat conformation (Figure 4c) was calculated to be 4.93 kcal/mol above the chair, B1 form. Using the Boltzmann relationship at 298 K and assuming no significant entropic differences, the relative amounts of the chair, B1; twist, B1; and chair, B2 forms of compound 7 at 298 K were calculated to be 94.36%, 5.62%, and 0.02%, respectively. Figure 7a,b depicts the two lowest energy conformations of 11-OH-α-HHC and their relationships with the REV map. In Figure 7a, it is clear that a portion of the C9 substituent of the chair, B1 form of 11-OH-α-HHC *protrudes* into the REV. This conformer, then, is
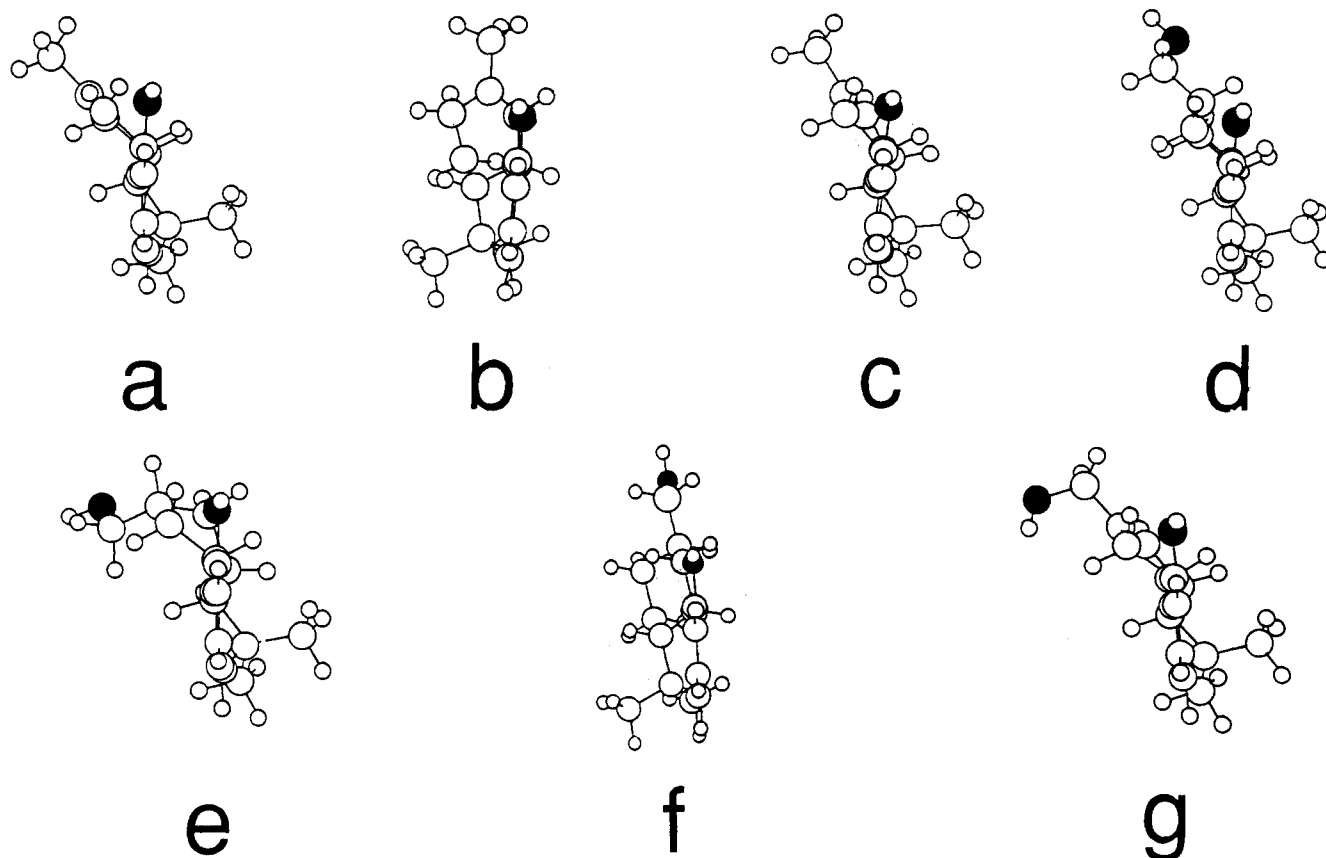
a b c d

e f g

Figure 2. Conformations as determined by MMP2(85) of (a) the half-chair, B1 conformer of (−)-Δ^9-THC (1, global minimum); (b) the half-chair, B2 conformer of (−)-Δ^9-THC (1); (c) the half-chair, B1 conformer of (−)-Δ^8-THC (2, global minimum); (d) the chair, B1 conformer of (−)-11-OH-β-HHC (3, global minimum); (e) the twist, B1 conformer of (−)-11-OH-β-HHC (3); (f) the chair-B2 conformer of (−)-11-OH-β-HHC (3); and (g) the half-chair, B1 conformer of (−)-11-OH-Δ^8-THC-DMH (4, global minimum). Table II provides further information on these accessible conformers of the active cannabinoids 1–4. All conformers are depicted without their C3 side chains. The perspective of the carbocyclic ring (ring A) in each is viewed in the direction parallel to the vector from C2 to C10b. The oxygens in each are shown here as blackened circles.
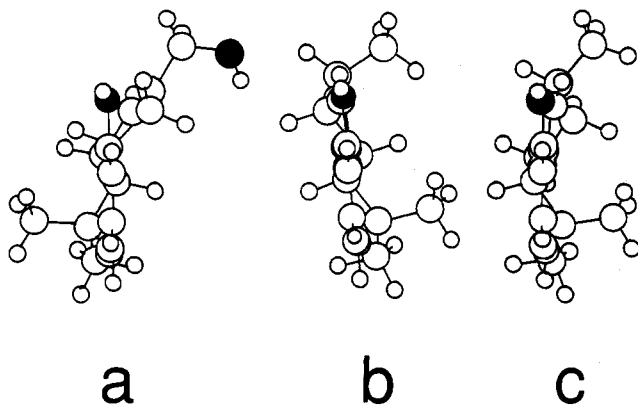

a b c

Figure 3. Conformations as determined by MMP2(85) of (a) the half-chair, B1 conformer of (+)-11-OH-Δ^8-THC-DMH (5, global minimum); (b) the half-chair, B1 conformer of (9S,6aR)-$\Delta^{10,10a}$-THC (6, global minimum); and (c) the half-chair 2, B1 conformer of (9S,6aR)-$\Delta^{10,10a}$-THC (6). Table II provides additional information on these accessible conformers of the inactive cannabinoids 5 and 6. See Figure 2 for further details.

not shaped properly to fit at the site of action. However, Figure 7b illustrates that the higher energy twist, B1 conformer is shaped properly to clear the REV region. The relation between the REV and the representation of each of these two conformers at their van der Waals radii (not shown here) was consistent with the results depicted in Figure 7. Thus, the reduced activity of 11-α-OH-HHC may be due to the fact that only a higher energy (twist,

B1) conformer (5.62% calculated abundance at 298 K) is shaped properly to fit at the site of action.

Benzofuran Cannabinoid (8). Compound 8 is notably structurally different from compounds such as (−)-Δ^9-THC (1), possessing, for example, a dihydrofuran instead of a dihydropyran central ring. Yet, compound 8 does possess elements of the cannabinoid pharmacophore,[3] a phenolic hyroxyl and a side chain in positions analogous to those in classical cannabinoids. Compound 8 has been reported to exhibit a marked activity in the pigeon drug discrimination assay for cannabinoid activity ($ED_{50} = 0.17$ mg/kg).[16,17] In the course of their comparison of the structure of 8 with that of (−)-Δ^9-THC (1), Mechoulam et al.[16] measured a nonbonded torsion angle (C8–C1– –C3′–OH) to be analogous to the τ value defined by us previously (see Introduction).[8] These authors found that τ has a value of +78° in 8. The fact that an active cannabinoid possesses a large positive "τ" value is an apparent contradiction to the correlation we proposed between the nonbonded "protrusion" torsion angle, τ, and cannabinoid activity (i.e., $\tau < 0$, for active compounds).[8] In actuality, a contradiction does not exist here. Figure 7c shows that no portion of 8 protrudes into the REV. Thus, while it appears that 8 is quite structurally different from cannabinoids like (−)-Δ^9-THC, 8 posseses the essential features of the cannabinoid pharmacophore and is shaped properly to fit at the site of action. The apparent discrepancy in the sign of the nonbonded torsion angle reported by Mechoulam et al.[16] arises from the attempt to define a τ
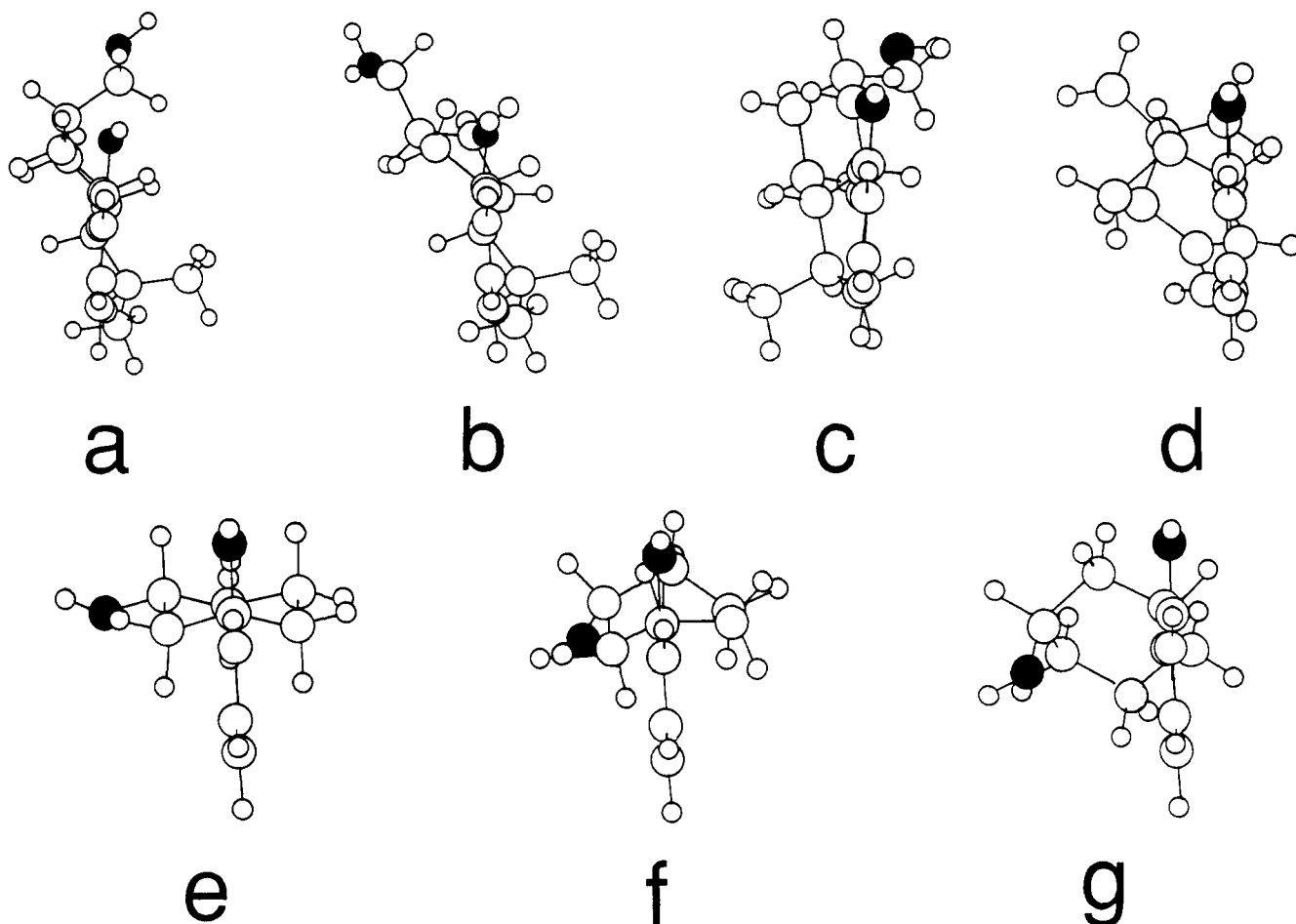
Figure 4. Conformations as determined by MMP2(85) of (a) the chair, b1 conformer of (-)-11-OH-α-HHC (7, global minimum); (b) the twist, B1 conformer of (-)-11-OH-α-HHC (7); (c) the chair, B2 conformer of (-)-11-OH-α-HHC (7); (d) the global-minimum conformer of 8; (e) the eq–eq, chair conformer of CP-47,497 (9, global minimum); (f) the eq–eq, twist conformer of CP-47,497 (9); and (g) the ax–ax, chair conformer of CP-47,497 (9). Table II provides additional information about the accessible conformers of compounds 7–9. See Figure 2 for further details.

value for a molecule which does not possess the same spatial relationship of the fused rings as that in the (-)-Δ⁹-THC-like cannabinoids for which τ was defined.[8] This finding illustrates one of the pitfalls in using geometrical parameters from nonrelated structures as criteria for activity.

CP-47,497 (9). Both rats and pigeons trained to discriminate (-)-Δ⁹-THC exhibit profound generalization to the flexible, nonclassical cannabinoid CP-47,497(9).[17] Boltzmann calculations at 298 K using the relative energies of the accessible conformers of 9 (see Table II and Experimental Section) reveal that greater than 99% of the molecules of 9 exist in a conformation in which the carbocyclic ring is in a chair conformation with the alcohol and aromatic rings occupying equatorial positions (eq–eq, chair form; Figure 4e). For the eq–eq, chair form of 9, a rotational energy study of the relative orientations of the two rings revealed that the carbocyclic and aromatic rings in this compound can exist in two minimum-energy positions relative to one another (C1–C10b–C10a–C10 = –117° and 63°). Inspection of the spatial relationship between the REV and the atoms of each minimum-energy rotamer contoured at its atomic van der Waals radii (not shown here), however, revealed that *neither rotamer was able to clear the REV*. However, a rotational energy study revealed that, at a small cost in energy (0.48 kcal/mol, no barrier), 9 can achieve a C1–C10b–C10a–C10 value (-78.2°) that allows the molecule to clear the REV and fit properly at the site of action. This circumstance is illustrated in

Figure 7d. In one of the higher minimum-energy forms of 9 (eq–eq, twist; abundance <0.1% at 298 K; see Table II), the molecule possesses a portion which protrudes into the REV. However, in another higher minimum-energy form (ax–ax, chair; abundance <0.1% at 298 K), no portion of the molecule protrudes into the REV. Since the eq–eq, chair form of 9 is by far its most predominant conformer at 298 K, the ability of a rotamer of this form to clear the REV with only a small energy expenditure may explain why 9 is an active cannabinoid.

Assumptions Used in REV Construction. Several assumptions are implicit in the calculation of the REV presented here: (1) Activity in a behavioral assay (drug discrimination) correlates with binding at the [³H]CP-55,940-labeled cannabinoid receptor. We have used the results of an *in vivo* assay for cannabinoid activity here because there are, at present, a very limited number of cannabinoid compounds that have been assayed for receptor binding. Both Devane et al.[20] and Herkenham et al.[21] have reported that the relative potencies of cannabinoids to inhibit [³H]CP-55,940 binding to the cannabinoid receptor parallel their activities in behavioral assays, as well as their abilities to regulate adenylate cyclase *in vitro*. Recently, Compton et al.[22] have reported that a high degree of correlation exists between the binding affinity of a series of structurally diverse cannabinoids at the [³H]CP-55,940-labeled cannabinoid receptor and the activities of these compounds in several behavioral phar-
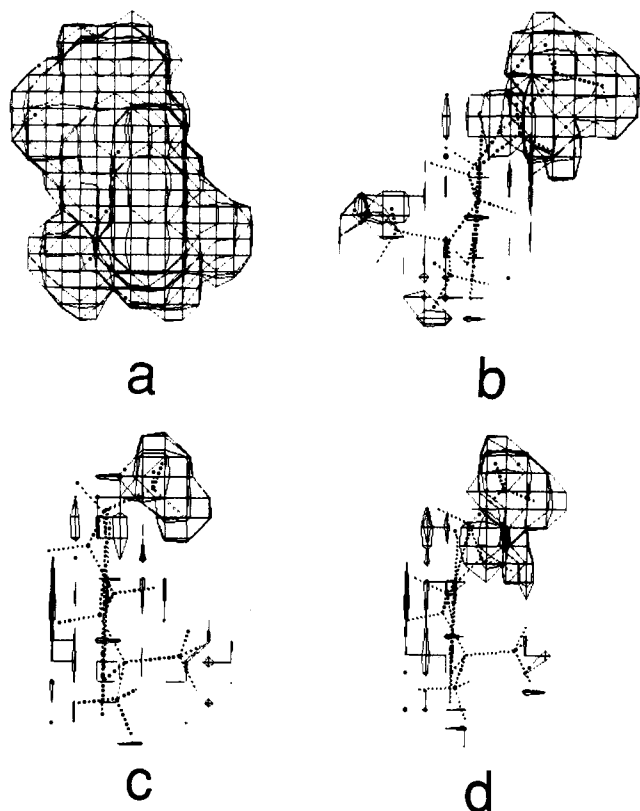
a   **b**

c   **d**

Figure 5. (a) The excluded volume map calculated by super-imposing the accessible conformers of (-)-Δ⁹-THC (Figures 2a and b), (-)-Δ⁸-THC (Figure 2c), (-)-11-OH-β-HHC (Figures 2d–f), and (-)-11-OH-Δ⁸-THC-DMH (Figure 2g). Here the excluded volume map is viewed along the direction of the vector from C2 to C10b. (b–d) The unique volumes (black cages) associated with (b) the half-chair, B1 (global minimum) conformer of (+)-11-OH-Δ⁸-THC-DMH (5); (c) the half-chair, B1 (global minimum) conformer of (9S,6aR)-Δ¹⁰,¹⁰ᵃ-THC (6); and (d) the half-chair 2, B1 conformer of (9S,6aR)-Δ¹⁰,¹⁰ᵃ-THC (6). Here the conformers (without side chains) are represented as stick drawings. See Figure 2 for details on the perspective of these drawings.
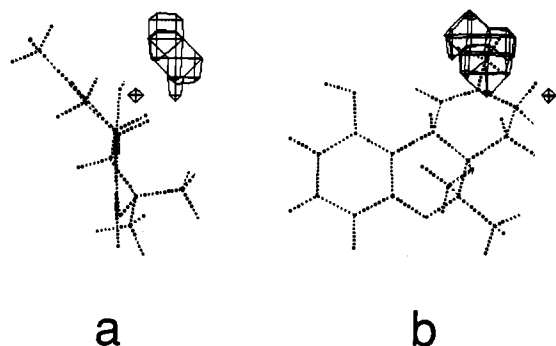

a   **b**

Figure 6. The receptor essential volume (REV) regions (black cages) as calculated from the accessible conformers of molecules 1–6. Here the REV regions are shown in relation to the global-minimum conformer of (-)-Δ⁹-THC (1). In part a the molecule is viewed along the direction of the vector from C2 to C10b and in part b the molecule is viewed from the bottom face looking toward the top face [here the phenol ring (ring C) is to the left and the carbocyclic ring (ring A) is to the right]. The C3 side chain of the molecule has been removed.

macological assays for cannabinoid activity including the drug discrimination model. (2) Changes in activity that accompany molecular modification in the cannabinoids can be directly attributed to changes in the ability of the cannabinoid to interact at its receptor. The metabolism, pharmacokinetics, and lipophilicity of drug molecules are important factors that should be considered in studies
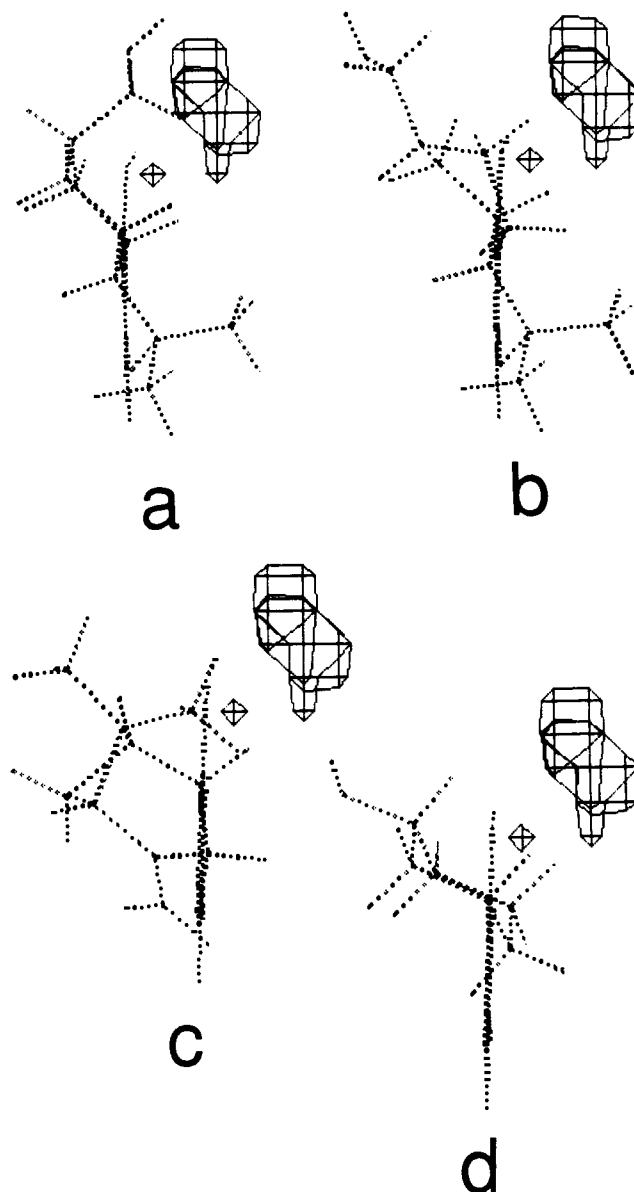

a   **b**

c

d

Figure 7. (a) The chair, b1 conformer of (-)-11-OH-α-HHC (7, global minimum) (b) the twist, B1 conformer of (-)-11-OH-α-HHC (7), (c) the single accessible conformer of 8, and (d) a rotamer of the eq–eq, chair conformer of CP-47,497 in which C1–C10b–C10a–C10 equals –78.2° are shown in relation to the REV regions. All molecules are shown without their side chains. See Figure 2 for details on the perspective of these drawings.

that involve the establishment of a correlation between molecular structure and activity using *in vivo* assays (as we have used in this study). Metabolic studies of the cannabinoids have indicated that there is no metabolic requirement for the production of psychoactivity in cannabinoids such as (-)-Δ⁹-THC.[23–25] Furthermore, it has been reported that differences in the penetration and in the distribution of cannabinoids in the central nervous system do not correlate with activity.[26,27] Thus, the assumption that changes in activity upon molecular modification can be attributed to changes in the ability of the molecule to interact at its receptor seems reasonable. (3) All of these compounds interact in analogous orientations at the receptor and therefore can be unambiguously superimposed upon one another. This assumption is supported by the very close structural similarity among the compounds studied and the presentation of the pharmacophoric pattern in essentially the same structural

elements in all compounds. Yet, it must be emphasized that the alignment rule used here to construct the REV is in itself simply a hypothesis concerning the relative orientations of these compounds at their site of action.[28]

Objections to mapping of the type we have peformed here come from the fact that the REV map is a static representation, yet protein structures (which commonly function as receptors) are flexible and possess dynamic mobility.[29] Our conformational studies of the fused ring cannabinoids, however, indicate that rather minimal steric differences between cannabinoids can eliminate activity.[8] These results, in turn, suggest that flexibility is limited at the site of action of these compounds.

It is important to emphasize that the ability of an accessible conformer of an analog to clear the REV region calculated here is a necessary but not a sufficient condition to produce activity, i.e., it is but one element of the cannabinoid pharmacophore. Cannabinoids must possess other features in addition to the steric feature delineated here in order to produce activity. Among these other requirements are the presence of a phenyl group hydroxyl at C1 and a side chain of adequate length at C3.[3] All of the subject molecules here possessed these additional pharmacophoric features.

Alternative Hypothesis. Mechoulam et al.[30] recently proposed a hypothesis alternative to the one explored here. In this hypothesis, loss of activity is proposed to result from an *improperly oriented* C9 substituent, i.e., a certain orientation of the C9 substituent is *necessary* for a specific interaction at the cannabinoid receptor. In this hypothesis, then, cannabinoids that cannot orient their C9 substituent properly will not achieve this specific interaction and will be rendered inactive. On the basis of the compounds discussed in this paper, this alternative hypothesis seems quite plausible. However, the fact that 9-nor-Δ^8-THC, which possesses no C9 substituent with which to interact at the receptor, still exhibits activity[31] argues against the alternative hypothesis. On the other hand, the activity of 9-nor-Δ^8-THC is consonant with the central hypothesis tested here, because the 9-nor compound possesses the other essential features of the cannabinoid pharmacophore and, in addition, possess no atoms that protrude into the REV region.

Conclusions

Our calculations imply that, at the cannabinoid receptor, a region of steric interference exists. In relation to the structure of the binding cannabinoid, this region is located near the top of the carbocyclic ring in the bottom face of the molecule. The results reported here should serve as an additional element to the cannabinoid pharmacophore and prove useful in the design of new cannabinoid analogs prior to synthesis.

Experimental Section

Structure Building, Optimization, and Conformational Analysis. In order to construct an REV using the molecules in Chart I and then explore its utility using the molecules in Chart II, it was first necessary to study all accessible energy minima of the fused ring conformers in these molecules. The crystal structure of Δ^9-THC acid B was used as the starting geometry for each cannabinoid.[32] The MODIFY facility within the Chem-X molecular modeling system[33] was used to delete unnecessary atoms and to add necessary ones at standard bond lengths and bond angles.[34] The initial phase of the characterization of each cannabinoid involved the elucidation of the various conformations that each compound may assume and the elucidation of the

relative probability of each conformation. The structure of each cannabinoid was optimized by using the method of molecular mechanics as encoded in the MMP2(85) program.[35] In the MM2 force field, the inclusion of special parameters to account for lone pairs on oxygens in ethers and alcohols is necessary. Without inclusion of these lone pairs, for example, one does not get the proper geometry for the methyl group in dimethyl ether.[35] Therefore, lone pairs (type = 20) were explicitly included in each optimization for all ether, alcohol, and phenyl group hydroxyl oxygens in the work presented here.

In order to ascertain if any other minimum-energy conformations of the fused-ring structure of each cannabinoid were possible, MMP2(85) dihedral or torsion angle driver studies were performed.[35,36] In this method, one or a combination of torsion angles is chosen as a reaction coordinate and is driven incrementally through a range of values, all other internal degrees of freedom being optimized at each increment.[37] The dihedral or torsion angle driving method has been commonly employed when conformational interconversions for large organic molecules have been studied by molecular mechanics. There are pitfalls associated with the torsion angle driving technique, as documented by Burkert and Allinger.[37] These pitfalls are associated largely with the identification of transition state geometries. The emphasis in the work presented here is on the identification of minima.

The selection of torsion angles to be driven to accomplish conformational interconversion depends on the molecule to be studied. There is very limited conformational flexibility in the fused-ring structures of compounds 1–8; in fact, there is *at most* one other minimum-energy conformation of each nonaromatic ring in these structures. In order to identify other minimum-energy conformations in the fused-ring structure of compounds 1–8, two torsion angles were driven (one in the carbocyclic ring and one in the dihydropyran ring (dihydrofuran ring in 8). The identification of the appropriate torsion angle for the carbocyclic ring driver studies varied with the type of carbocyclic ring present. For the tetrahydrocannabinol structures, the C10a–C6a–C7–C8 torsion angle (in (−)-Δ^9-THC, 1, and in (9S,6aR)-$\Delta^{10,10a}$-THC, 6), or the C10–C10a–C6a–C7 torsion angle (in the Δ^8-THCs 2, 4, 5) was identified, while for the hexahydrocannabinol structures 3 and 7, the C6a–C7–C8–C9 torsion angle was identified. For the benzofuran 8, the C4–C3–C2–C1 torsion angle was identified (see numbering system for 8 in Chart II), while for CP-47,497 (9), the C6a–C7–C8–C9 and the C6a–C10a–C10–C9 torsion angles were chosen. For all subject cannabinoids which contain dihydropyran rings, the C10b–C4a–O5–C6 torsion angle was chosen for the torsion angle studies of ring B. Dihedral or torsion angle studies of the carbocyclic and dihydropyran rings were conducted by driving the appropriate angle(s) (in which the central bond(s) is (are) endocyclic) using small increments (1°–10°) over a range of approximately 120° in order to accomplish each conformational interconversion. Minimum-energy fused-ring conformers identified by the torsion angle driver studies were considered to be accessible if their final steric energies were within 6 kcal/mol of the global-minimum-energy structure.[19]

For each minimum-energy fused-ring conformer identified above, separate torsion angle driver studies of phenyl group hydroxyls, CH$_2$OH groups, and alcohol groups were performed. Because the orientation in space of the phenolic hydroxyl at C1 is believed by us[9,38] and by others[39] to be a further determinant of cannabinoid activity, it was important to verify that all of the compounds studied possessed phenolic hydroxyls that could exist in the same relative minimum-energy orientations in space. To study other possible minimum-energy positions for the phenyl group hydroxyl in compounds 1–9, the C2–C1–O–H (C6'–C1'–O–H in 8) torsion angle was driven. For the CH$_2$OH group in compounds 3–5 and 7, the C10–C9–C11–O torsion angle was driven, while for the alcohol group in compound 9, the C10–C9–O–H torsion angle was driven.

Because CP-47,497 (9) lacks the dihydropyran (or dihydrofuran) rings of compounds 1–8, the carbocyclic and aromatic rings of compound 9 can rotate relative to one another. In order to determine if any other minimum-energy relative ring positions were possible for compound 9, we performed torsion angle driver studies of the C1–C10b–C10a–C10 torsion angle. Since compound 9 can exist in a form in which both the alcohol at C9 and the

phenyl ring at C10a are equatorial (eq–eq form) and in a form in which both the alcohol at C9 and the phenyl ring at C10a are axial (ax–ax form), we performed ring drives for both forms.

Dihedral or torsion angle studies of all rotatable groups or rotatable rings in 1–9 were conducted by driving the appropriate angle (in which the central bond is exocyclic) using 10° increments over a range of 360°. Minimum-energy conformers whose final steric energies were within 6 kcal/mol of the energy of the global-minimum conformer were considered accessible.[19]

REV Construction. All of the subject molecules in this study possess two pharmacophoric elements: phenyl group hydroxyl oxygens at C1 and side chains of acceptable length at C3.[3] Because these pharmacophoric elements are associated with an aromatic ring, they do not change their relative positions upon conformational interconversion of the fused-ring structure. Therefore, all fused-ring conformers of the subject molecules that were identified as accessible were included in the REV construction. Since more than one minimum-energy rotamer can exist for each accessible fused-ring conformer, the lowest energy rotamer of each accessible fused-ring conformer was included in the construction of the REV. For example, in (–)-Δ⁸-THC (2) only one accessible fused-ring conformer was identified while two minimum-energy phenyl group hydroxyl positions were identified. For the REV construction, therefore, the lower energy phenyl group hydroxyl form of (–)-Δ⁸-THC was used.

Before the calculation of the REV, the side chain of each accessible fused-ring conformer was removed to facilitate the visualization of the fused-ring structure. This removal is acceptable since the focus of this study is on the fused-ring structures of the cannabinoids and not on their side chains. Furthermore, since all of the cannabinoids in our set possess side chains of acceptable length,[3] the molecular feature that is the determinant of activity/inactivity in the present study is *not* the side chain.

The elements of the cannabinoid pharmacophore were used as guides to the alignment of all the studied molecules. The aromatic rings of each conformer were superimposed by aligning the rings at C1, C2, and C10b and the phenyl group hydroxyl oxygen. The global minimum-energy conformer of (–)-Δ⁹-THC (1) was used as the template to which all other accessible conformers in the set were fit, using a fitting procedure in Chem-X,[33] with equal weight being given to each position.

The general procedure of Sufrin et al.[4] was used to calculate the REV. Using the MAP facility within Chem-X and a density of 2 points per Å, the van der Waals volume map of each accessible fused-ring conformer of compounds 1–4 (active cannabinoids) and of compounds 5 and 6 (inactive cannabinoids) was calculated. The union of the van der Waals volume maps of all accessible conformers of the active cannabinoids (compounds 1–4) allowed construction of the excluded volume map (Figure 5a). The excluded volume map represents that volume available at the site of action as indicated by the *volume requirements of active molecules*.

Each accessible fused-ring conformer of the inactive compounds (5 and 6) was examined for unique volume requirements not associated with the excluded volume map. This was done by performing a logical NOT operation for the van der Waals volume map of each inactive conformer with the excluded volume map. The result of this operation is the volume unique to the inactive cannabinoid (i.e., the volume not occupied by the set of active molecules). This procedure was carried out for each accessible fused-ring conformer of each inactive cannabinoid (Figure 5b–d). Finally, the intersection of the unique volume portions of each inactive analog yielded the region of *unique volume overlap for all inactive cannabinoids*, the receptor essential volume (REV, Figure 6). The REV can be interpreted as a region occupied by the receptor or site of action and therefore not available for occupancy by other molecules.[4]

Application of REV. After removal of their side chains, all accessible conformers of compounds 7–9 (see Chart II) were screened for their ability to clear the REV calculated above. To this end, each atom of each conformer was drawn at its van der Waals radius. Any conformer that possessed one or more atoms that exhibited protrusion into the REV region when these atoms were contoured at their van der Waals radii was judged unable to fit at the site of action.

Accessible Conformers. Tetrahydrocannabinols (1, 2, 4, 5, and 6). Our calculations revealed that, in the global minimum-energy conformer of (–)-Δ⁹-THC (1), (–)-Δ⁸-THC (2), (–)-11-OH-Δ⁸-THC-DMH (4), (+)-11-OH-Δ⁸-THC-DMH (5), and (9S,6aR)-Δ¹⁰,¹⁰ᵃ-THC (6), the cyclohexene ring (ring A) and the dihydropyran (ring B) rings exist in half-chair conformations. Values of all ring A cyclohexene ring torsion angles are given in Table I. In the half-chair dihydropyran conformation (conformation B1), the axial C6(α) methyl group is on the same side of the molecule as H10a and is much closer to H10a than is the other methyl group. The substituents on C6–C6a are staggered with respect to each other. The optimized dihydropyran (ring B) angle, C10a–C6a–C6–O, value was 59°–63° for 1, 2, 4, and 6, and –62° for the (+)-enantiomer, 5. The half-chair conformations of the cyclohexene and dihydropyran rings in 1, 2, 4, 5, and 6 are in keeping with our previous calculations[8,38] and with NMR solution studies of 1.[7,16,41]

Dihedral driver studies of the THCs 1, 2, and 4–6 revealed that only the carbocyclic ring in 6 was capable of adopting a second conformation. (The dihydropyran ring here remains in the B1 conformation). This second conformation of the cyclohexene ring in 6 was another slightly distorted half-chair (called here half-chair 2). The existence of a half-chair 2, B1 conformation for 6 is in keeping with the two half-chair conformations expected for cyclohexene itself.[36] The lack of a second half-chair conformation in 1, 2, 4, and 5 is to be expected due to the fusion of the carbocyclic ring to the dihydropyran ring.

The only tetrahydrocannabinol that was found to have a second accessible dihydropyran conformation was (–)-Δ⁹-THC (1). In this conformer, ring A remains in a half-chair while ring B assumes a boat conformation such that the C6 methyl group in the axial position nearly eclipses H6a along the C6–C6a bond. We have called this boat conformation of ring B the B2 conformation. The C10a–C6a–C6–O torsion angle of ring B in the half-chair, B2 conformation of 1 was found to be 29°. The UV spectrum of 2,3-dihydropyran immediately after irradiation by a pulse of laser IR light shows the presence of two species that have been reported to correspond to the same two local energy minima as we have identified here for the dihydropyran ring, the half-chair and the boat.[40]

Torsion angle driver studies of the phenyl group hydroxyl in the tetrahydrocannabinols revealed that there are two minimum-energy positions for the phenyl group hydroxyl in 1, 2, 4, 5, and 6. In position I, the phenyl group hydroxyl is essentially in the plane of the aromatic ring with the hydrogen of the phenyl group hydroxyl pointing away from the carbocyclic ring. In position II, this hydrogen points toward the carbocyclic ring. For the global-minimum-energy conformers of 1, 2, and 4, Position I values were 6°–8° and position II values were 166°–168°, while for the (+)-enantiomer 5 they were –6° and –166°. Compound 5, however, is capable of achieving phenyl group hydroxyl positions analogous to each of those of 1, 2, and 4 with expenditure of less than 0.64 kcal/mol (no barrier). For the global-minimum-energy conformer of 6, the position I value was 0° and the position II value was 160°.

Torsion angle driver studies of other minimum-energy positions of the CH₂OH group in compounds 4 and 5 revealed three minimum-energy positions for this group. For the global-minimum-energy conformers of molecules 4 and 5, the three minima (in order of increasing energy) corresponded to C10–C9–C11–O values of –60°, 70°, and –172° for 4 and 60°, –70°, and 173° for 5. Tables I and II include a summary of the conformational results for the tetrahydrocannabinols.

Hexahydrocannabinols (3 and 7). In the global-minimum-energy conformer of the HHCs (3 and 7), the cyclohexane ring (ring A) was found to exist in a chair conformation while the dihydropyran ring was found to exist in the half-chair (conformation B1) described above for the tetrahydrocannabinols. Our results for this chair, B1 form of 3 and 7 are consistent with our earlier calculations for another pair of HHC compounds[8] and with solution NMR studies of one HHC isomer.[41] The global-minimum-energy conformer for 11-OH-β-HHC (chair, B1), in which the CH₂OH substituent at C9 is equatorial, was found to be 1.67 kcal/mol lower in steric energy than the global-minimum-energy conformer of its α epimer, 11-OH-α-HHC (chair, B1). This amount compares well with the 1.70 kcal/mol stabilization

determined experimentally for equatorial versus axial methyl-cyclohexane in solution.[36]

A second accessible minimum-energy fused-ring conformation was found for each HHC (3 and 7) in which the carbocyclic ring exists in a twist conformation while the dihydropyran ring remains in a half-chair conformation (twist, B1). For 11-OH-β-HHC (NL-105, 3), the twist, B1 conformer was 5.53 kcal/mol above the global-minimum conformation. This energy difference is quite similar to the 5.36 kcal/mol difference calculated for the chair versus twist forms of cyclohexane.[37] On the other hand, the second minimum conformer in 11-OH-α-HHC (NL-106, 7), which also corresponds to a twist, B1 conformation, was only 1.67 kcal/mol above that of its chair, B1 conformation. This result for chair, B1 versus twist, B1 forms of 7 is consistent with our earlier molecular mechanics calculations of the relative energies of chair, B1 versus twist, B1 forms of 11α-HHC. Here we found the relative energy difference to be 1.72 kcal/mol.[8]

Finally, for both 3 and 7, a third accessible minimum-energy fused-ring conformation was identified. In this conformation, the carbocyclic ring exists in a chair conformation while the dihydropyran ring exists in a boat conformation (chair, B2). For compound 3, this chair, B2 conformer was 4.81 kcal/mol above the global-minimum conformer, while in 7 this conformer was 4.93 kcal/mol above the global minimum.

Rotational studies of the phenyl group hydroxyl in 3 and 7 revealed the same two minima as identified above for the tetrahydrocannabinols. Studies of other minimum-energy positions of the CH_2OH group in 3 and 7 revealed three minima: 173°, 63°, and −57° for 3 and −170°, −60°, and 70° for 7. Tables I and II include a summary of the conformational results for the hexahydrocannabinols.

Benzofuran (8). Our calculations revealed that compound 8 possesses only one minimum-energy fused-ring conformation. The phenyl group hydroxyl in 8 was found to exist in two minimum energy (C6'−C1'−O−H) positions: 1° and −179°. Compound 8, however, can achieve phenyl group hydroxyl positions analogous to those of the tetra- and hexahydrocannabinols by the expenditure of 0.25 kcal/mol (no barrier). See Table II for a summary of the results for 8.

CP-47,497 (9). CP-47,497 can be thought of as a phenyl- and alcohol-substituted cyclohexane. In one chair form of the cyclohexane ring, both the alcohol and the phenyl groups occupy equatorial positions (eq−eq). If the cyclohexane ring is converted to its other chair form, the alcohol and phenyl groups of CP-47,497 then both occupy axial positions (ax−ax). A phenyl substituent alone on a cyclohexane ring usually prefers to be equatorial by 3.01 kcal/mol, a much stronger preference than that found for a methyl group (1.70 kcal/mol).[36] Our MMP2(85) studies of 9 showed that the phenyl and alcohol groups together preferred equatorial positions (i.e., the eq−eq, chair form) by 5.84 kcal/mol. In 9, as in phenylcyclohexane, the equatorial phenyl was calculated to prefer a conformation in which its plane contains the bond to the isohydrogen.[36] When the phenyl and alcohol substituents of 9 are axial, our MMP2(85) calculations revealed that the conformation with the phenyl plane perpendicular to the CH bond was the better one. This same conformation is predicted for an axial phenyl group substituent on cyclohexane due to transannular repulsion.[36]

Torsion angle driver studies of the cyclohexane ring in 9 revealed that this ring can exist also in a twist conformation. Only the eq−eq, twist form was found to be accessible, however, with a steric energy of 5.73 kcal/mol above the eq−eq, chair form. The phenyl group hydroxyl in 9 was found to exist in two minimum-energy positions: C2−C1−O−H = 0° and 179°. Compound 9, however, can achieve C2−C1−O−H values analogous to those of the tetra- and hexahydrocannabinols by the expenditure of 0.14 kcal/mol (no barrier). For the eq−eq chair form of 9, three minimum-energy positions of the OH corresponded to C10−C9−O−H values (in order of increasing energy) of 65°, 172°, and −61°.

For the eq−eq, chair form of 9, a rotational energy study of the relative positions of the cyclohexane and aromatic rings revealed minima at C1−C10b−C10a−C10 = −117° and 63°. The minimum at 63° was found to be 0.77 kcal/mol higher in final steric energy than the minimum at −117°. For the eq−eq, twist form, the minima occurred at −131° and at 58°. Here the minimum at 58°

was 0.57 kcal/mol higher in final steric energy than the minimum at −131°. For the ax−ax, chair form of compound 9, the drive revealed two minima at C1−C10b−C10a−C10 values of −67° and 143°.

Our results for the eq−eq, chair form of 9 are consistent with an earlier molecular mechanics study of this compound using the MM1 force field.[18] In this study, the steric energy as a function of the relative rotation of the cyclohexane and aromatic rings was also studied. Two minima that differed in steric energy by 0.7 kcal/mol (estimated graphical value) were found. Our calculations predict the same two minima with a 0.77 kcal/mol difference in final steric energy.

References

(1) Mechoulam, R.; Gaoni, Y. Recent Advances in the Chemistry of Hashish. *Fortschr. Chem. Org. Naturst.* 1967, 25, 175–213.
(2) Gaoni, Y.; Mechoulam, R. The Isolation and Structure of Delta-1-Tetrahydrocannabinol and Other Neutral Cannabinoids from Hashish. *J. Am. Chem. Soc.* 1971, 93, 217–224.
(3) Razdan, R. K. Structure–Activity Relationships in the Cannabinoids. *Pharm. Rev.* 1986, 38, 75–149.
(4) Sufrin, J. R.; Dunn, D. A.; Marshall, G. R. Steric Mapping of the L-Methionine Binding Site of ATP: L-Methionine S-Adenosyltransferase. *Mol. Pharmacol.* 1981, 19, 307–313.
(5) Binder, M.; Edery, H.; Porath, G. Δ7-Tetrahydrocannabinol, A Non-Psychotropic Cannabinoid: Structure–Activity Considerations in the Cannabinoid Series. In *Marihuana Biological Effects: Analysis, Metabolism, Cellular Responses, Reproduction and Brain*; Nahas, G. G., Paton, W. D. M., Eds.; Pergamon Press: Oxford, 1979; pp 71–80.
(6) Mechoulam, R.; Feigenbaum, J. J.; Lander, N.; Breuer, A.; Conroe, P.; Järbe, T. U. C.; Hiltunen, A. J. Stereospecificity and Stereoselectivity of Cannabinoid Action. In *Marihuana: An International Research Report*; Chesher, G., Conroe, P., Musty, R., Eds.; Australian Government Publishing Service: Canberra, 1988; pp 243–251.
(7) Kriwacki, R. W.; Makriyannis, A. The Conformational Analysis of Δ8- and Δ9,11-Tetrahydrocannabinols in Solution using High Resolution Nuclear Magnetic Resonance Spectroscopy. *Mol. Pharmacol.* 1989, 35, 495–503.
(8) Reggio, P. H.; Greer, K. V.; Cox, S. M. The Importance of the Orientation of the C9 Substituent to Cannabinoid Activity. *J. Med. Chem.* 1989, 32, 1630–1635.
(9) Reggio, P. H.; Seltzman, H. H.; Compton, D. R.; Prescott, W. R.; Martin B. R. Investigation of the Role of the Phenolic Hydroxyl in Cannabinoid Activity. *Mol. Pharmacol.* 1990, 38, 854–862.
(10) Järbe, T. U. C.; Swedberg, M. D. B.; Mechoulam, R. A. Repeated Tests Procedure to Assess Onset and Duration of the Cue Properties of (−)-Δ9-THC, (−)-Δ8-THC-DMH, and (+)-Δ8-THC. *Psychopharmacology* 1981, 75, 152–157.
(11) Henriksson, B. G.; Johansson, J. O.; Järbe, T. U. C. Δ9-Tetrahydrocannabinol Produced Discrimination in Pigeons. *Pharmacol. Biochem. Behav.* 1975, 3, 771–774.
(12) Järbe, T. U. C.; Henriksson, B. G.; Ohlin, G. C. Δ9-THC as a Discriminative Cue in Pigeons: Effects of Δ8-THC, CBD, and CBN. *Arch. Int. Pharmacodyn.* 1977, 228, 68–72.
(13) Järbe, T. U. C.; Hiltunen, A. J.; Lander, N.; Mechoulam, R. Cannabimimetic Activity (Δ1-THC Cue) of Cannabidiol Monomethyl Ether and Two Stereoisomeric Hexahydrocannabinols in Rats and Pigeons. *Pharmacol. Biochem. Behav.* 1986, 25, 393–399.
(14) Mechoulam, R.; Feigenbaum, J. J.; Lander, N.; Segal, M.; Järbe, T. U. C.; Hiltunen, A. J.; Conroe, P. Enantiomeric Cannabinoids: Stereospecificity of Psychotropic Activity. *Experientia* 1988, 44, 762–764.
(15) Järbe, T. U. C.; Hiltunen, A. J.; Mechoulam, R.; Srebnik, M.; Breuer, A. Separation of the Discriminative Stimulus Effects of Stereoisomers of Δ2- and Δ3-Tetrahydrocannabinols in Pigeons. *Eur. J. Pharmacol.* 1988, 156, 361–366.
(16) Mechoulam, R.; Breuer, A.; Järbe, T. U. C.; Hiltunen, A. J.; Glaser, R. Cannabimimetric Activity of Novel Enantiomeric Benzofuran Cannabinoids. *J. Med. Chem.* 1990, 33, 1037–1043.
(17) Järbe, T. U. C.; Mathis, D. A. Discriminative Stimulus Functions of Cannabinoids/Cannabimimetics. In *Drug Discrimination: Applications to Drug Abuse Research*; NIDA Research Monograph 116; Glennon, R. A., Järbe, T. U. C., Frankenheim, J., Eds.; U.S. Dept. of Health and Human Services: Washington, D.C., 1991; pp 75–99.
(18) Melvin, L. S.; Johnson, M. R.; Harbert, C. A.; Milne, G. M.; Weissman, A. A Cannabinoid Derived Prototypical Analgesic. *J. Med. Chem.* 1984, 27, 67–71.

(19) Reggio, P. H.; McGaughey, G. B.; Odear, D. F.; Seltzman, H. H.; Compton, D. R.; Martin, B. R. A Rational Search for the Separation of Psychoactivity and Analgesia in Cannabinoids. *Pharmacol. Biochem. Behav.* **1991**, *40*, 479–486.

(20) Devane, W. A.; Dysarz, F. A., III; Johnson, M. R.; Melvin, L. S.; Howlett, A. C. Determination and Characterization of a Cannabinoid Receptor in Rat Brain. *Mol. Pharmacol.* **1988**, *34*, 605–613.

(21) Herkenham, M.; Lynn, A. B.; Little, M. D.; Johnson, M. R.; Melvin, L. S.; DeCosta, B. R.; Rice, K. C. Cannabinoid Receptor Localization in Brain. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 1932–1936.

(22) Compton, D. R.; Bridgen, D. T.; Rice, K. C.; DeCosta, B.; Martin, B. R. THC Receptor SAR-Correlations Between Ligand Binding and *In Vivo* Activity. International Cannabis Research Society Meeting, Keystone, CO, 1992.

(23) Martin, B. R.; Dewey, W. L.; Harris, L. S.; Beckner, J.; Wilson, R. S.; May, E. L. Marijuana-like Activity of New Synthetic Tetrahydrocannabinols. *Pharmacol. Biochem. Behav.* **1975**, *3*, 849–853.

(24) Martin, B. R.; Agurell, S.; Krieglstein, J.; Rieger, H. Perfusion of the Isolated Rat Brain with (14C)-.Delta.-1-Tetrahydrocannabinol. *Biochem. Pharmacol.* **1977**, *26*, 2307–2309.

(25) Carney, J. M.; Balster, R. C.; Martin, B. R.; Harris, L. S. Effects of Systemic and Intraventricular Administration of Cannabinoids on Schedule-Controlled Responding in the Squirrel Monkey. *J. Pharmacol. Exp. Ther.* **1979**, *210*, 399–404.

(26) Binder, M.; Witteler, F.-J.; Schmidt, B.; Franke, I.; Bohnenberger, E.; Sanderman, H., Jr. Triglyceride/Phospholipid Partitioning and Pharmacokinetics of Some Natural and Semi-Synthetic Cannabinoids: Further Evidence for the Involvement of Specific Receptors in the Mediation of the Psychotropic Effects of Delta-1-THC and Delta-6-THC. In *The Cannabinoids: Chemical Pharmacologic and Therapeutic Aspects*; Agurell, S., Dewey, W. L., Willette, R. B., Eds.; Academic Press: New York, 1983; pp 709–727.

(27) Thomas, B. F.; Compton, D. R.; Martin, B. R. Characterization of the Lipophilicity of Natural and Synthetic Analogs of Δ^9-Tetrahydrocannabinol and its Relationship to Pharmacological Potency. *J. Pharmacol. Exp. Ther.* **1990**, *255*, 624–630.

(28) Westkaemper, R. B.; Glennon, R. A. Approaches to Molecular Modeling Studies and Specific Application to Serotonin Ligands and Receptors. *Pharmacol. Biochem. Behav.* **1991**, *40*, 1019–1031.

(29) Humblet, C.; Marshall, G. R. Three Dimensional Computer Modeling as an Aid to Drug Design. *Drug Dev. Res.* **1981**, *1*, 409–434.

(30) Mechoulam, R.; Devane, W. A.; Glaser, R. Cannabinoid Geometry and Biological Activity. In *Marijuana/Cannabinoids Neurobiology and Neurophysiology*; Murphy, L.; Bartke, A., Eds.; CRC Press: Boca Raton, FL, 1992; pp 1–33.

(31) Wilson, R. S.; May, E. L. 9-Nor-Delta-8-THC, a Cannabinoid of Metabolic Interest. *J. Med. Chem.* **1974**, *17*, 475–476.

(32) Rosenqvist, E.; Ottersen, T. The Crystal and Molecular Structure of Δ^9-Tetrahydrocannabinolic Acid B *Acta. Chem. Scand.* **1975**, *B29*, 379–384.

(33) Chem-X, developed and distributed by Chemical Design, Ltd., Oxford, England.

(34) Mitchell, A. L.; Cross, L. C., Eds. *Tables of Interatomic Distances and Configurations in Molecules and Ions*; Special Publication 11; The Chemical Society: London, 1958; Special Publication 18, 1965.

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

(36) Burkert, U.; Allinger, N. L. *Molecular Mechanics*; ACS Monograph Series 177; American Chemical Society: Washington, DC, 1982.

(37) Burkert, U.; Allinger, N. L. Pitfalls in the Use of the Torsion Angle Driving Method for the Calculation of Conformational Interconversions. *J. Comput. Chem.* **1982**, *3*, 40–46.

(38) Reggio, P. H.; Mazurek, A. P. A Molecular Reactivity Template for Cannabinoid Activity. *J. Mol. Struc. (Theochem.)* **1987**, *149*, 331–343.

(39) Semus, S. F.; Martin, B. R. A Computergraphic Investigation Into the Pharmacological Role of the THC-Cannabinoid Phenolic Moiety. *Life Sciences* **1990**, *46*, 1781–1785.

(40) Garcia, D.; Grunwald, E. Pulsed IR Laser Study of Half-Chair to Boat Interconversion of 2,3-Dihydropyran. *J. Am. Chem. Soc.* **1980**, *102*, 6407–6411.

(41) Archer, R. A.; Boyd, D. B.; Demarco, P. V.; Tyminski, I. J.; Allinger, N. L. Structural Studies of Cannabinoids. A Theoretical and Proton Magnetic Resonance Analysis. *J. Am. Chem. Soc.* **1970**, *92*, 5200–5206.