Pharmacology Biochemistry & Behavior, Vol. 40, pp. 479–486. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/91 $3.00 + .00

A Rational Search for the Separation of Psychoactivity and Analgesia in Cannabinoids

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REGGIO, P. H., G. B. McGAUGHEY, D. F. ODEAR, H. H. SELTZMAN, D. R. COMPTON AND B. R. MARTIN. *A rational search for the separation of psychoactivity and analgesia in cannabinoids.* PHARMACOL BIOCHEM BEHAV **40**(3) 479–486, 1991.—The compound 9-beta-hydroxy-hexahydrocannabinol [(−)-9β-OH-HHC] was designed to fit a combined theoretical profile of an analgesic cannabinoid (equatorial alcohol at C-9, phenol at C-1 and a C-3 side chain) with reduced psychoactivity (axial C-9 substituent which protrudes into the α face). (−)-9β-OH-HHC was synthesized by the addition of methyl Grignard to 9-oxo-11-nor-HHC. Its α epimer was obtained by the regiospecific epoxide ring opening of 9α, 10α-epoxy-HHC acetate. (−)-9β-OH-HHC and (−)-9α-OH-HHC were each evaluated in a battery of tests in mice and were found to be 10–25 times less potent than (−)-trans-Δ^9-tetrahydrocannabinol (Δ^9-THC) in all tests including the tail flick test for antinociception (analgesia). Molecular mechanics calculations [MMP2(85)] revealed that, in the global minimum energy conformation of (−)-9β-OH-HHC, the axial methyl at C-9 protrudes into the α face of the molecule, while the axial hydroxyl at C-9 in (−)-9α-OH-HHC protrudes into this same face. These calculations also identified a higher energy carbocyclic ring (*twist*) conformer of each in which there is no protrusion of a C-9 substituent of the carbocyclic ring into the α face. The minimal activity of both compounds is attributed to these higher energy forms. It is concluded that protrusion of a C-9 substituent into the α-face of the molecule is associated with reduced cannabinoid analgesia, as well as with reduced cannabinoid psychopharmacological activity.

Analgesia Antinociception Cannabinoid Conformational analysis Psychopharmacological activity Structure-activity relationship 9α-Hydroxy-hexahydrocannabinol 9β-Hydroxy-hexahydrocannabinol

THE separation of cannabinoid antinociceptive (analgesic) activity from cannabinoid psychopharmacological activity has been long sought. In the 70s, Wilson and May's postulate that the analgesic activity of Δ^9-THC was due to its 11-hydroxy metabolite spurred much interest in cannabinoid analgesia. Wilson and May supported their conclusion by the observation that the 9-nor derivative, which cannot be transformed into the 11-hydroxy metabolite, lacks significant analgesic activity, but exhibits dog ataxia and cardiovascular profiles nearly identical to Δ^9-THC (25). Wilson et al. (26) then synthesized and evaluated the 9-nor-9α-OH and 9-nor-9β-OH analogs of hexahydrocannabinol (HHC) (see Fig. 1). They found that, although both the 9-nor-9α and the 9-nor-9β analogs possessed psychopharmacological activity in dogs and in mice (the former compound being 4–5 times less potent that the latter), only (−)-9-nor-9β-HHC possessed analgesic activity. Interestingly, (−)-9-nor-9β-OH-HHC was equipotent with morphine and Δ^9-THC in the mouse hot plate (antinociception) test, possessing an ED_{50} of 1.6 mg/kg, while (−)-9-nor-9α-OH-HHC was inactive at up to 50 mg/kg. These authors concluded that 11- or 9β-hydroxylation was a prerequisite for the analgesic activity but not necessarily for the psychopharmacological activity of the cannabinoids. The pharmacological results for 9-nor-9α- and 9-nor-9β-HHC suggested the possibility of selective separation of cannabimimetic responses. Unfortunately, such separation was in the wrong direction—with psychopharmacological activity remaining for cannabinoids whose analgesic potency had disappeared (8).

More recent SAR literature on analgesic cannabinoids has been concerned primarily with the enhancement of analgesia based upon a fixed cannabinoid nucleus. Johnson and Melvin,

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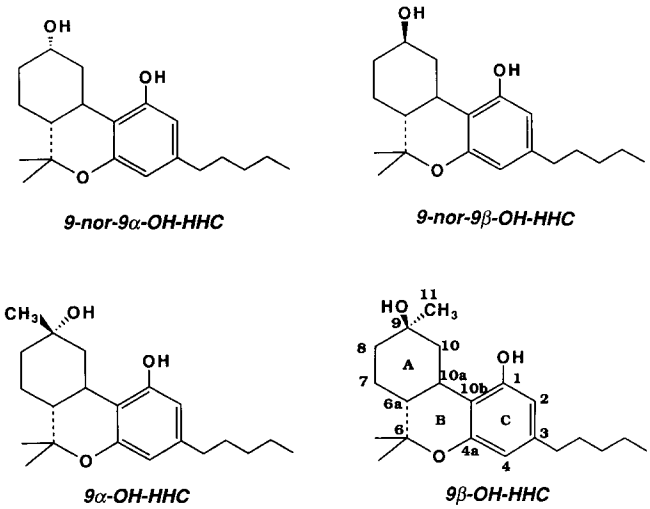

FIG. 1. Drawings of (−)-9-nor-9α-OH-HHC, (−)-9-nor-9β-OH-HHC, (−)-9α-OH-HHC, and (−)-9β-OH-HHC. The numbering system illustrated for (−)-9β-OH-HHC is one commonly used for the cannabinoids.

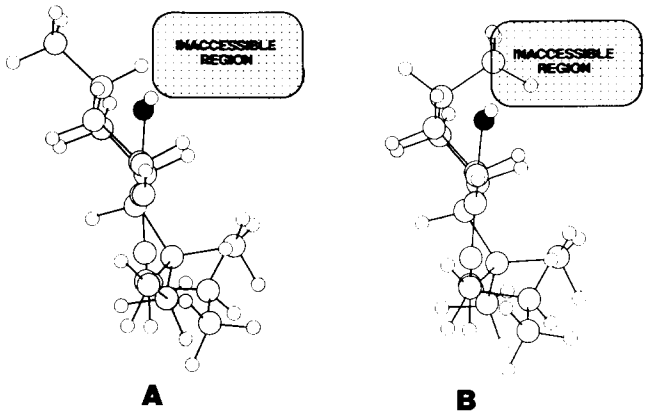

FIG. 2. Side views of (A) (−)-Δ^9-THC (active) and (B) (−)-Δ^7-THC (inactive) with the hypothesized region of steric inaccessibility illustrated. The phenolic oxygen is shown as a blackened circle. Here the perspective of Ring A is viewed in the direction of the vector from C2 to C10b. The side chain, therefore, is forward, and the carbocyclic ring (Ring A) is in the back. In (A), all atoms clear the inacessible region, while in (B), some atoms of the C-9 methyl group protrude into the inaccessible region.

for example, worked with the (−)-9-nor-9β-OH-HHC structure and made modifications to this structure which improved analgesia (10). However, they were not able to simultaneously abolish psychoactivity in any of the analgesics they produced. Milne and Johnson have postulated that "HHC interacts at a receptor site by a three-point contact, the three binding sites being the equatorial alcohol, phenol, and C-3 side chain" (14).

In previous work on the molecular requirements for cannabinoid psychopharmacological activity, Reggio et al. found what seems to be a steric requirement for activity (22). For cannabinoids which differ from Δ^9-THC only in the position or absence of a double bond in the carbocyclic ring (Ring A, see Fig. 1), those cannabinoids that possess a C9 substituent that protrudes into the top (β) face of the molecule (i.e., above the plane of the paper in Fig. 1) are active, while those that possess a C9 substituent that protrudes into the bottom (α) face (i.e., below the plane of the paper in Fig. 1) are inactive. This protrusion of the C9 substituent was quantified by measuring a nonbonded torsion angle, the "protrusion torsion angle" (O-C1--C9-C11). Active cannabinoids were found to have negative values for this angle, while inactive cannabinoids possessed positive values. These findings on the protrusion of the C9 substituent were interpreted as evidence that there may be an inaccessible region near the top of the carbocyclic ring in the cannabinoids which is occupied by atoms of the receptor or site of action. Therefore, only cannabinoids with structures that do not protrude into this region will fit properly at the site of action. Figure 2 provides an illustration of the hypothesized spatial relationship between the shape of an active or inactive cannabinoid and this inaccessible region.

In an effort to design a cannabinoid analgesic agent with reduced psychoactive liability, we sought to combine in one molecule those molecular characteristics that have been hypothesized to be necessary for cannabinoid analgesia with a characteristic that has been hypothesized to produce cannabinoid psychopharmacological *inactivity*. (−)-9β-OH-HHC (Fig. 1) possesses an equatorial alcohol at C9, a phenol at C1, and a C3 side chain, thus fitting Milne and Johnson's analgesia hypothesis (14). (−)-9β-OH-HHC differs from (−)-9-nor-9β-OH-HHC, however, by possessing an axial methyl group at the 9 position in addition to an equatorial hydroxyl at this same position. This axial methyl group at C9 should protrude into the bottom (α) face of the molecule thus resembling psychopharmacologically inactive molecules (22).

(−)-9α-OH-HHC, the α epimer of (−)-9β-OH-HHC (see Fig. 1), does not fit the Milne and Johnson hypothesis because its hydroxyl group at C9 is axial rather than equatorial. This axial hydroxyl group should protrude into the bottom (α) face of the molecule, thus resembling psychopharmacologically inactive cannabinoids (22).

We describe here the minimum energy accessible conformers of (−)9β-OH-HHC and of (−)9α-OH-HHC as determined by molecular mechanics calculations, the synthesis and pharmacological evaluation of (−)9β-OH-HHC and (−)9α-OH-HHC, and the implications these pharmacological results have for cannabinoid SAR.

METHOD

Conformational Analysis

The crystal structure of Δ^9-THC acid B (23) was used as the starting geometry for both 9α and 9β-OH-HHC. The Modify facility within the CHEM-X molecular modeling system (6) was used to delete unnecessary atoms and to add necessary ones at standard bond lengths and bond angles (15). The side chain in both molecules was shortened from pentyl to propyl in order to keep the molecule small enough for ab initio quantum mechanical calculations in a later stage of the study. Such a modification is acceptable, since the focus of this study is on the fused ring structure of each molecule and not on the length of its side chain.

The structures of both 9α and 9β-OH-HHC were optimized by using the method of molecular mechanics as encoded in the MMP2(85) program (1). Lone pairs of electrons were included in each optimization for the ether, alcohol, and phenol oxygens. In order to ascertain if any other minimum energy conformations of the fused ring structure exist, MMP2(85) dihedral driver studies were performed (4). A dihedral driver study of the carbocyclic ring (Ring A, see Fig. 1) in each molecule was con-

ducted by driving the C6a-C7-C8-C9 angle from 53° to −47° in increments of 2°–8° for 9α-OH-HHC and from 52° to −48° in increments of 3°–10° for 9β-OH-HHC. A dihedral driver study of the pyran ring (Ring B, see Fig. 1) was also performed on the minimum energy conformer of 9α-OH-HHC and of 9β-OH-HHC. In this study, the C10b-C4a-O-C6 angle was driven from 19° to −81° in increments of 2°–10° for 9α-OH-HHC and from 19° to −80° in increments of 2°–10° for 9β-OH-HHC.

For each minimum energy conformer of the fused ring structure of both 9α-OH-HHC and 9β-OH-HHC with steric energy less than 9–10 kcal/mol above the global minimum, we performed separate dihedral driver studies of the minimum energy positions of the 9α-hydroxyl or the 9β-hydroxyl group and of the phenol group. For the 9α-hydroxyl group in 9α-OH-HHC and for the 9β-hydroxyl group in 9β-OH-HHC, the C10-C9-O-H angle was driven around 360° in 10° increments. For the phenol group in each, the C2-C1-O-H angle was driven around 360° in 10° increments. For each minimum energy conformer identified by molecular mechanics, we measured the protrusion torsion angle (O-C1--C9-O for 9α-OH-HHC or O-C1–C9-C11 for 9β-OH-HHC) using the Calculate Geometry facility within the CHEM-X molecular modeling system (6).

Synthesis of 9α-OH-HHC and 9β-OH-HHC

Two approaches to the preparation of the title compound and its alpha epimer (9α-OH-HHC) were examined. All transformations were conducted on single enantiomers having the natural configurations at C-10a and C-6a. The first approach was the hydride opening of the epoxides of Δ^8-, Δ^9-, $\Delta^{9,11}$-THC acetates. Regiospecific epoxide ring opening of 9α, 10α-epoxy-HHC acetate (13) with $LiAlH_4$ following the reported method (18) provided 9α-OH-HHC with a ^1H NMR spectrum that agreed with the literature. The α- and β-epoxides of Δ^8-THC acetate were prepared (18), separated (27) and reduced with $LiAlH_4$. The α-epoxide afforded 9α-OH-HHC, while the β-epoxide gave 8β-OH-HHC (18), both products being the result of *trans*diaxial ring opening.

The stereospecificity of the chemical epoxidation of $\Delta^{9,11}$-THC acetate has not been reported, while the microsomal epoxidation was reported to occur from the less hindered β-face of the olefin (2). This suggested that the chemical epoxidation might also afford the β-epoxide, which could then be employed as a precursor to the β-alcohol. The epoxidation of $\Delta^{9,11}$-THC acetate with m-chloroperoxybenzoic acid afforded 9,11-epoxyhexahydrocannabinol acetate (20) that exhibited GC and reverse-phase HPLC chromatograms that showed two marginally separable components in a 4:1 ratio. The components were not separable by silica gel chromatography as either the acetate or as the free phenol. Reduction of the epoxide acetate mixture with $LiAlH_4$ afforded 9α-OH-HHC and 9β-OH-HHC in a 4:1 ratio as determined by HPLC versus authentic 9α-OH-HHC (see above) and confirmed by ^1H NMR. The preponderance of the α-alcohol indicates preferred epoxidation of $\Delta^{9,11}$-THC from the more hindered α-face.

9β-OH-HHC was synthesized by the addition of methyl Grignard to 9-oxo-11-nor-HHC following the reported procedure (19). This report did not identify the stereochemistry at the 9 position of the product(s). The alcohols were separated (2:1 β:α) in 48% total yield by silica gel chromatography (8% acetone-toluene) along with 34% of recovered starting material. The major product was identified as 9β-OH-HHC by its HPLC retention time difference versus the known 9α-OH-HHC and its ^1H NMR, ^{13}C NMR, two-dimensional heteronuclear correlation spectrum, IR, elemental and high-resolution mass spectral analyses (Table 1) that were consistent with an isomer of the latter. Especially revealing was the ^{13}C NMR, which was essentially the same for both isomers except in the region of the structural difference about C-9. Chemical shifts of the proximate carbons 7, 8, 9, 10, 10a and especially 9-Me were significantly different for the two isomers. The large upfield shift of the axial methyl in 9β-OH-HHC versus the corresponding equatorial methyl in 9α-OH-HHC is also consistent with the 9-Me chemical shift differences of similar 9-hydroxy-HHC analogs (3).

The Grignard reaction went to completion when the acetate of 9-oxo-11-nor-HHC was employed following the literature procedure (9). Here, HPLC analysis indicated a 1:1 mixture of the α- and β-alcohols. The product obtained by crystallization of this mixture was shown to be the 9β-isomer by ^1H NMR and HPLC comparison to the material prepared above. However, the literature assigned the product of the crystallization to be the 9α-isomer based on its dehydration ($POCl_3$, pyridine) to the endocyclic Δ^8-THC olefin in analogy with the behavior of similar steroid alcohols. When we examined the dehydration of each al-

TABLE 1

CMR CHEMICAL SHIFT ASSIGNMENTS*

Carbon	9α-OH-HHC	9β-OH-HHC	HHC
1	157.1†	157.1†	157.2†
2	108.2	108.2	108.2
3	142.4	142.6	142.6
4	109.6	109.5	109.6
4a	156.0†	155.8†	155.8†
10b	111.1	110.5	111 ‡
6	76.9	76.9	77.0
6a	50.1	50.6	50.3
7	24.6	26.4	28.8
8	40.2	41.7	36.5§
9	69.6	70.9	33.6
10	43.4	44.8	39.8
10a	31.4	33.7	36.5
6α-Me	19.4	19.3	19.3
6β-Me	28.2	28.2	28.1
9-Me	32.1	26.9	23.0
α	36.1	36.1	36.1
β	31.5	31.5	31.6
γ	32.2	32.2	32.3
δ	23.1	23.1	23.2
ϵ	14.2	14.2	14.3

*^1H NMR and CMR spectra were run on a Bruker WM 250 spectrometer in acetone-d_6. α–ϵ refer to the pentyl side chain carbons from the benzylic methylene to the terminal methyl respectively. Assignments in part per million downfield from Me_4Si; δ (acetone-d_6-CD_3) + 29.8 ppm.

†These signals may be reversed. ‡Scale value not digital output. §Possible overlap.

9β-OH-HHC: ^1H NMR (acetone-d_6): δ 0.87 (t, 3H, J=6.8 Hz, ϵ CH3), 1.03 (s, 3H, 6α-Me), 1.32 (s,9-Me), 1.33 (s, 6β-Me), 1.79 (m, 2H, 8-CH_2), 2.37 (t, 2H, J=7.6 Hz, α CH_2), 2.52 (dt, 1H, J=2.7, 11.5 Hz, 10a-H), 3.32 (td, 1H, J=2.4, 12.3 Hz, 10α-H), 3.62 (s, 1H, D_2O exchangeable, 9-OH), 6.07 (d, 1H, J=1.6 Hz, H-4), 6.19 (d, 1H, J=1.6 Hz, H-2), 8.27 (s, 1H, ArOH). HRMS: Calculated for $C_{21}H_{32}O_3$: 332.2351; Found 332.2353. Elemental Analysis: Calculated for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70; Found C, 75.59; H, 9.92.

Gas chromatography (2% OV-17, 235°C): 9β-OH-HHC (equatorial 9-OH) silylates faster than 9α-OH-HHC (axial 9-OH) in DMF consistent with the configurational assignments.

TABLE 2

RESULTS OF CONFORMATIONAL ANALYSIS OF 9α-OH-HHC

Ring A	Ring B	Phenol C2-C1-O-H (degrees)	Alcohol C10-C9-O-H (degrees)	Δ FSE (kcal/mol)	Protrusion Torsion Angle O-C1--C9-O
Chair	B1	8	−57	0.00	57
		8	−177	0.51	54
		8	63	1.00	51
		168	−175	0.30	51
		165	−75	0.92	50
		168	85	1.51	48
Twist	B1	6	−57	5.51	−8
		6	58	5.54	−9
		6	180	5.75	−9
		168	−180	6.08	−10
		166	−62	6.09	−9
		168	62	6.74	−11
Chair	B2	2	−62	5.02	105
		2	50	5.87	101
		2	178	7.09	104
		162	177	7.09	108
		167	−68	7.34	110
		165	55	7.90	107
Twist	B2	−1	−63	10.18	80

TABLE 3

RESULTS OF CONFORMATIONAL ANALYSIS OF 9β-OH-HHC

Ring A	Ring B	Phenol C2-C1-O-H (degrees)	Alcohol C10-C9-O-H (degrees)	Δ FSE (kcal/mol)	Protrusion Torsion Angle O-C1--C9-C11
Chair	B1	8	60	0.00	47
		8	−60	0.25	49
		8	180	0.31	48
		168	−179	0.86	45
		167	−59	0.86	45
		168	61	0.96	45
Twist	B1	6	64	2.71	−17
		5	178	2.86	−17
		6	−56	3.60	−12
		165	65	3.62	−19
		166	177	3.67	−20
		167	−55	4.51	−15
Chair	B2	2	−56	5.11	100
		1	−179	5.18	99
		1	57	6.05	99
		−178	−180	6.82	104
		−167	55	7.14	105
		174	−65	7.23	106
Twist	B2	2	64	9.59	23

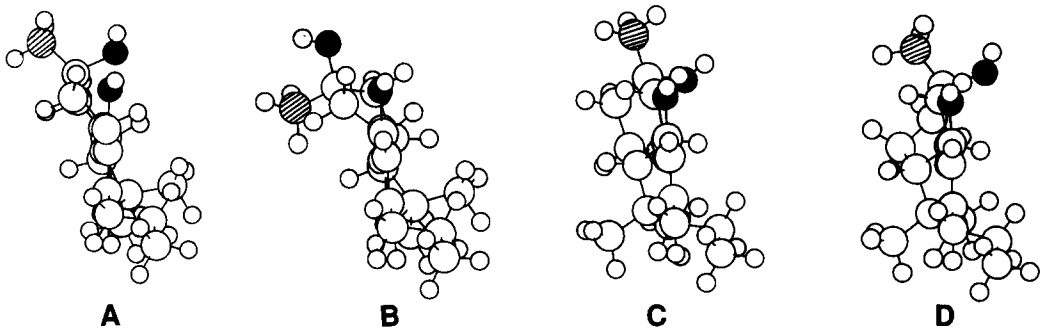

FIG. 3. Side views of all minimum energy fused ring conformers of (−)-9α-OH-HHC (with propyl side chains): (A) *chair, B1*; (B) *twist, B1*; (C) *chair, B2*; and (D) *twist, B2*. The carbon of the methyl substituent at C-9 is shown here as a hatched circle; the phenolic and 9α-OH oxygens are shown as blackened circles. 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) is in the back. See Table 2 for the relative final steric energies (ΔFSEs) of these conformers.

cohol by the same method, both the α- and β-alcohols gave only the endocyclic olefin (GC and ^{1}H NMR). The melting point of the previously prepared racemic material was 162–163°C. The melting point of our crystalline, single enantiomer β-isomer was 187–191°C, while the melting point of the chromatographed solid α-isomer, which could not be crystallized from the literature solvent, was 67–69°C. These observations argue that the material previously prepared (9) was the β- and not the α-isomer.

Drug Preparation and Administration

Male ICR mice (22–30 g) obtained from Dominion Laboratories (Dublin, VA) were maintained on a 14:10-hour light:dark cycle, and received food and water ad lib. Micellular suspensions of the drugs were prepared for injection by the procedure of Olson et al. (16). 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 route.

Behavioral and Pharmacological Evaluations

The analogs were evaluated in a battery of tests in mice which has been shown to be predictive of cannabinoid effects (12,21). Mice were acclimated to the observation room (ambient temperature 20–24°C) overnight. Rectal temperature was determined on partially restrained mice by a thermistor probe (inserted 25 mm) and a telethermometer (Yellow Springs Instrument Co., Yellow Springs, OH) prior to vehicle or drug administration. Additionally, the latency period (in s) was measured on a standard tail flick apparatus (7). The heat lamp of the tail flick apparatus was maintained at an intensity sufficient to produce control latencies of 2–4 s. Mice received tail vein injections (0.1 ml/10 g) and were placed into individual photocell activity chambers 5 min later. For a 10-min period, locomotor activity was measured in a Digiscan Animal Activity Monitor (Omnitech Electronics, Inc., Columbus, OH) as the number of interruptions of 16 photocell beams per chamber, and expressed as % control activity. Tail flick latency was assessed again at 20 min after the injection, and the increase in the latency period (s) for each mouse was recorded. To avoid tail injury, an automatic heat lamp cut-off time of 10 s was used. Rectal temperature was measured again at 60 min after the injection, and the difference between pre- and postinjection values (Δ°C) was calculated for each animal. Mice were evaluated for ring immobility 1.5 h after the injection utilizing a slight modification of the method of Pertwee (17). Each mouse was placed onto a 5.5-cm ring for 5 min. The period of time during which the mouse remained motionless was measured during the 5-min period. This value was divided by 300 s and multiplied by 100 to obtain % immobility. Mice that did not remain on the ring at least 2.5 min prior to

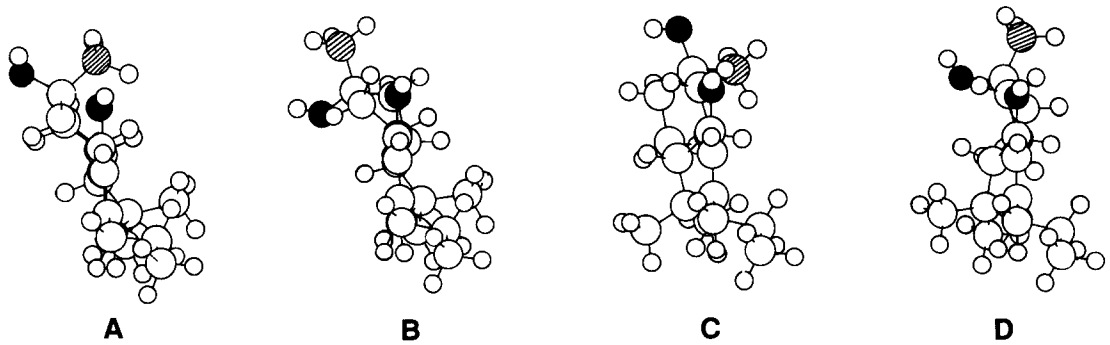

FIG. 4. Side views of all minimum energy fused ring conformers of (−)-9β-OH-HHC (with propyl side chains): (A) *chair, B1*; (B) *twist, B1*; (C) *chair, B2*; and (D) *twist, B2*. The carbon of the methyl substituent at C-9 is shown here as a hatched circle. The phenolic and 9β-OH oxygens are shown as blackened circles. See Table 3 for the relative final steric energies (ΔFSEs) of these conformers. See Fig. 3 for further details.

TABLE 4

PHARMACOLOGICAL EFFECTS OF TWO HEXAHYDROCANNABINOL ANALOGS

Analog	Measure	E_{max}	ED_{50} (mg/kg)	95% C.L.
9α-OH-HHC	Motor activity	72% inhibition	8.6	5.1–14
	Temperature	−5.2°C	27	14–50
	Tail-flick latency	100% MPE	26	13–48
	Ring-immobility	69% immobility	~104*	29–360
9β-OH-HHC	Motor activity	45% inhibition	not determined	not determined
	Temperature	−5.8°C	36	24–53
	Tail-flick latency	100% MPE	23	14–39
	Ring-immobility	38% immobility	16	10–27

*Value is estimated. See text for explanation.

five escapes failed to meet the minimum criteria of this evaluation, and data were disregarded.

Data Analysis

Depression of locomotor activity, hypothermia, and ring immobility were expressed as % control activity, Δ°C, and % immobility, respectively. Antinociception was calculated as the percent maximum possible effect (% MPE) based on the increase in the tail flick latency period (in s) for each mouse and the maximum possible test latency of 10 s (7). The MPE values (already defined in terms of a maximum effect of 100%) were converted to probit values and the MPE_{50} determined by unweighted least-squares linear regression analysis of the log dose versus probit plot. A theoretical maximum effect of a given drug on % control activity, Δ°C, or % immobility was calculated from double reciprocal analysis (1/effect versus 1/dose) as described by Tallarida and Murray (24). The fractional response for each dose of drug was calculated (based upon a maximum effect of 1.0 for each individual behavioral measure), converted to probit values, and the ED_{50} 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 to the vehicle control, and differences were considered significant at the $p<0.05$ level (two-tailed). The method of Litchfield and Wilcoxon (11) was used to determine confidence limits for the ED_{50}s.

RESULTS

Conformational Analysis

Figures 3 and 4 illustrate side views of each minimum energy fused ring conformer of 9α- and 9β-OH-HHC, respectively. Tables 2 and 3 summarize all of the conformational results. In the global minimum energy structure of both 9α- and 9β-OH-HHC, the cyclohexane ring (Ring A) exists in a *chair* conformation. The pyran ring (Ring B) assumes a conformation such that the axial C6 (α) methyl group is on the same side of the molecule as H_{10a} and is much closer to H_{10a} than is the other methyl group. The substituents on C6 and C6a are staggered with respect to one another. The optimized C10a-C6a-C6-O value was 63° for both 9α-OH-HHC and 9β-OH-HHC (see Fig. 1 for numbering system). We have called this pyran ring conformation *Conformation B1*. In both 9α- and 9β-OH-HHC, the phenolic hydrogen optimized at a position pointing away from the cyclohexane ring (Ring A), and the 9-hydroxyl hydrogen optimized at a position pointing towards the aromatic ring.

Other Ring Conformations

Dihedral driver studies of the cyclohexane ring (Ring A) revealed that a second minimum-energy conformation of the cyclohexane ring exists for both 9α-OH-HHC and 9β-OH-HHC. This minimum corresponds to a *twist* conformation. When Ring B, the phenol, and 9-OH groups are in their lowest energy forms, this *twist* conformation is 5.51 kcal/mol above the *chair* conformation in 9α-OH-HHC and 2.71 kcal/mol above the *chair* conformation in 9β-OH-HHC. The energy difference calculated for the *chair* versus *twist* forms of cyclohexane is 5.36 kcal/mol (5).

Dihedral driver studies of the pyran ring (Ring B) revealed that a second minimum energy conformation also exists for this ring in both 9α and 9β-OH-HHC. We have called this pyran ring conformation *Conformation B2*. In this second conformation, the C6 (β) methyl group in the axial position nearly eclipses H6a along the C6-C6a bond. The C10a-C6a-C6-O torsion angle was 19° for 9α-OH-HHC and 18° for 9α-OH-HHC.

Phenol Conformations

Dihedral driver studies of the phenol group revealed that there are two minimum energy positions for the phenol group in both 9α- and 9β-OH-HHC. In *Phenol Conformation I* (C2-Cl-O-H = 8° for the global minimum of 9α- and 9β-OH-HHC), the phenol group is essentially in the plane of the aromatic ring with the phenolic hydrogen pointing away from the cyclohexane ring. In *Phenol Conformation II* (C2-Cl-O-H = 168°), the phenolic hydrogen points towards the cyclohexane ring. These two phenol positions (or values near to these) were found not only for the global minimum conformation of the fused ring structures of 9α- and 9β-OH-HHC, but also for the higher energy ring conformers discussed previously (see Tables 2 and 3). These same two phenol positions have been found previously for Δ^9-THC and several other cannabinoids (22).

9-Hydroxyl Group Conformations

Dihedral driver studies of other minimum energy positions of the 9-hydroxyl group in 9α- or 9β-OH-HHC revealed that this group can exist in three minimum energy positions (see Tables 2 and 3).

Behavioral Pharmacology Evaluation

The ability of 9α-OH-HHC and 9β-OH-HHC to produce hypoactivity, hypothermia, antinociception (via tail flick), and cat-

alepsy (via ring immobility) was evaluated in the mouse following intravenous injection. The analogs were characterized in terms of their potency (ED_{50}) and their efficacy (E_{max}), and the data presented in Table 4.

These HHC analogs were weakly potent, and analysis of dose-response data proved difficult due to a limited number of active doses. Thus the determination of E_{max} and ED_{50} was essentially dependent upon results obtained at the 30- and 100-mg/kg doses. Regardless, standard techniques (see Martin et al., this volume) were used to determine E_{max} and ED_{50} values.

9α-OH-HHC, though not potent, presented a typically cannabinoid pharmacological profile. This analog was effective in all four measures, and produced maximum responses similar to that observed for Δ^9-THC (data not shown). This analog was nearly 10 times less potent than Δ^9-THC in the spontaneous activity measure, and approximately 20 times less potent in the temperature and tail flick procedures. The ED_{50} and E_{max} values for this analog on ring immobility must be interpreted cautiously. Although the analysis of the ring immobility data (three doses) estimated an E_{max} of 69%, the actual response observed at 100 mg/kg was only 36% ($\pm$SE = 5%). Since only half of the complete dose-response curve was obtained, the ED_{50} value was estimated to be 104 mg/kg.

9β-OH-HHC also exhibited a cannabinoid pharmacological profile. It was approximately 10, 15, and 25 times less potent than Δ^9-THC in the ring immobility, tail flick, and temperature assays, respectively. The ring immobility E_{max} (38%) was consistent with the observed effect at 100 mg/kg (45 $\pm$ 8%), which was not different from that obtained with 10 mg/kg Δ^9-THC. 9β-OH-HHC depressed locomotor activity, but not in a fashion typical of the cannabinoids, due to a lack of dose-responsiveness and the magnitude of the effect observed.

DISCUSSION

The pharmacological results suggest that there is no significant separation of pharmacological activity between 9α-OH-HHC and 9β-OH-HHC, which appear to be 10–25 times less potent than Δ^9-THC, and can be best described as cannabimimetic despite minor descrepancies.

In 9α-OH-HHC, the 9α-OH group protrudes into the bottom face of the molecule in the global minimum structure (*Chair, B1*: Protrusion Torsion Angle, O-Cl--C9-O = 57°, see Fig. 3A). According to the steric inaccessiblity hypothesis for cannabinoid psychopharmacological activity mentioned previously, this conformer would be expected to be unable to fit at the site of action. However, of the three other minimum energy fused ring conformations calculated for 9α-OH-HHC, the *twist, B1* form of 9α-OH-HHC (see Fig. 3B) is not only capable of clearing the hypothesized region of interference (O-C1--C9-O = −8° to −9°), but also of placing the 9α-OH group into the same general region of space that the 9β-OH group occupies in the global minimum form of 9β-OH-HHC (Fig. 4A). Thus this higher energy form (*twist, B1*) of 9α-OH-HHC should be able to fit into the hypothesized site and be able to present its alcohol and phenol functionalities in the same regions of space as in 9-nor-9β-OH-HHC (14). This higher energy form of 9α-OH-HHC may, then, be responsible for the minimal activity of 9α-OH-HHC.

Previously, we have assumed that only minimum energy conformers with energies within 3 kcal/mol of the energy of the global minimum conformer would be accessible at ordinary temperatures (22). However, our results for 9α-OH-HHC seem to indicate that even the *twist, B1* conformer which is at least 5.51 kcal/mol above the global minimum may be accessible. The amount of energy required for 9α-OH-HHC to adopt its *twist, B1* form might be compensated for by favorable interactions at the site of action.

9β-OH-HHC possesses an equatorial alcohol, a phenol, and a C3 side chain. Thus it possesses all the requirements hypothesized by Milne and Johnson to be necessary for cannabinoid analgesia (14). Yet our pharmacological results indicate that 9β-OH-HHC is minimally active as an analgesic. In its global minimum conformation, 9β-OH-HHC possesses an axial methyl which protrudes into the bottom face of the molecule (Protrusion Torsion Angle O-Cl--C9-C11 = 47°, see also Fig. 4A). One possible explanation for the minimal activity of 9β-OH-HHC is that, in this global minimum conformer (which is the most predominant form, Fig. 4A), the axial methyl at C9 produces steric interference at the site of action just as has been hypothesized for inactive cannabinoid psychopharmacological agents (22). The activity that 9β-OH-HHC exhibits, therefore, may be due instead to another of its fused ring conformers, the less prevalent *twist, B1* form which is at least 2.71 kcal/mol higher than the global minimum form (see Fig. 4B). In this *twist, B1* form, the C9 methyl group clears the hypothesized region of steric interference (i.e., does not protrude into the bottom face). Thus only a higher energy form of 9β-OH-HHC is shaped properly to fit at the site of action. This result may explain the low activity (rather than inactivity) exhibited by 9β-OH-HHC.

CONCLUSIONS

Our original goal of separating cannabinoid analgesic activity from cannabinoid psychopharmacological activity cannot be met by the inclusion of an axial C-9 substituent in cannabinoid analgesic molecules. If we assume that there are no significant differences in the pharmacokinetics or metabolism of 9α- and 9β-OH-HHC, then our results seem to indicate that the same steric accessibility requirement that has been postulated previously for cannabinoid psychopharmacological activity (22) also exists for cannabinoid antinociception (analgesic activity). Thus it appears that compounds that fit Milne and Johnson's three-point contact model must also clear a region of steric inaccessibility near the top of the carbocyclic ring in order to exhibit antinociception (analgesia).

ACKNOWLEDGEMENTS

This work was supported by grants DA-03934 and DA-03672 from the National Institute on Drug Abuse and by a Council on Undergraduate Research Academic-Industrial Undergraduate Research Partnership (AIURP) Fellowship from the American Cyanamid Company, Agricultural Research Division (G.B.M.). The computations were supported, in part, by a grant of computer time from the School of Chemistry, Georgia Institute of Technology.

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