Pergamon

Life Sciences, Vol. 56, No.s 23/24, pp. 2025-2032, 1995

0024-3205/95 $9.50 + .00

0024-3205(95)00185-9

THE DESIGN, SYNTHESIS AND TESTING OF DESOXY-CBD: FURTHER EVIDENCE FOR A REGION OF STERIC INTERFERENCE AT THE CANNABINOID RECEPTOR

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Summary

Cannabidiol CBD, a non-psychoactive constituent of marihuana, has been reported to possess essentially no affinity for cannabinoid CB1 receptor binding sites in the brain. Our hypothesis concerning CBD's lack of affinity for the cannabinoid CB1 receptor is that CBD is not capable of clearing a region of steric interference at the CB1 receptor and thereby not able to bind to this receptor. We have previously characterized this region of steric interference at the CB1 receptor [P.H. Reggio, A.M. Panu, S. Miles J. Med. Chem. 36, 1761-1771 (1993)] in three dimensions using the Active Analog Approach. We report here a conformational analysis of CBD which, in turn, led to the design of a new analog, desoxy-CBD. Modeling results for desoxy-CBD predict that this compound is capable of clearing the region of steric interference by expending 3.64 kcal/mol of energy in contrast to the 12.39 kcal/mol expenditure required by CBD. Desoxy-CBD was synthesized by condensation of 3-pentylphenol with p-mentha-2,8-dien-1-ol mediated by DMF-dineopental acetal. Desoxy-CBD was found to behave as a partial agonist in the mouse vas deferens assay, an assay which is reported to detect the presence of cannabinoid receptors. The compound produced a concentration related inhibition of electrically-evoked contractions of the mouse vas deferens, possessing an IC_{50} of 30.9 nM in this assay. Taken together, these results support the hypothesis of the existence of a region of steric interference at the CB1 receptor. While the energy expenditure to clear this region was too high for the parent compound, CBD, the removal of the C6' hydroxyl of CBD produced a molecule (desoxy-CBD) able to clear this region and produce activity, albeit at a reduced level.

Key Words: cannabidiol, desoxy-cannabidiol, vas deferens

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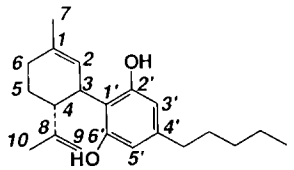

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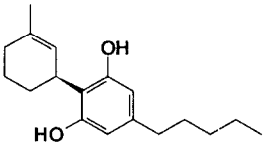

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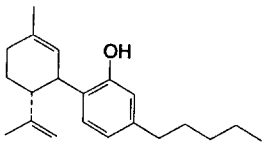

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Cannabidiol (CBD,**1**) is a non-psychoactive constituent of *cannabis sativa L.* (The structure of CBD, as well as the monoterpene numbering scheme commonly employed for CBD are illustrated above.) Both hashish and marihuana are derived from cannabis and have been used for centuries for their medicinal and psychotomimetic properties (1). CBD has been reported to possess potentially important pharmacological properties as an anti-epileptic (2), an anxiolytic (3), and as an antidyskinetic agent (4). While these effects of CBD may be receptor mediated, they are thought not to be cannabinoid CB1 receptor mediated as, for example, CBD has essentially no affinity for cannabinoid CB1 binding sites in the brain (5) and it exhibits no detectable inhibitory effect on electrically-evoked contractions of the mouse vas deferens at concentrations up to 100 nM (5). The mouse vas deferens is a preparation which is thought to contain cannabinoid CB1 receptors that can mediate an inhibitory effect of cannabinoid receptor agonists on electrically-evoked contractions (5-8).

In traditional cannabinoid SAR, both a phenolic hydroxyl at C1 and an alkyl side chain at C3 (C2' and C4' respectively in the monoterpene numbering system) are considered requirements for CB1 receptor mediated activity (9). CBD possesses both of these pharmacophoric requirements, yet has no affinity for the CB1 receptor. Recently, we have demonstrated that an additional requirement for CB1 mediated activity is that the molecule must be shaped properly to avoid a sterically inaccessible region at the CB1 receptor located near the top of the carbocyclic ring in the bottom face of the molecule (10). This region is termed a Receptor Essential Volume (REV) region and is hypothesized to represent an area of space occupied by the receptor itself. Studies reported here suggest that CBD is unable to clear this REV region and therefore unable to bind at the CB1 receptor and produce effects mediated by this receptor. Results from our CBD study led us to design two CBD analogs and to test each for their ability to clear the REV. One of these analogs, desoxy-CBD, **3**, proved able to meet our REV criteria and was subsequently synthesized and tested. In this paper, we report our conformational study of CBD and our resultant design of desoxy-CBD, as well as, the synthesis and pharmacological testing of desoxy-CBD.

Methods

Computational Studies. *Conformational Analysis.* The crystal structure of Δ^9-THC acid B was used as the starting geometry for each cannabinoid (11). The MODIFY facility within the Chem-X molecular modeling system was used to delete unnecessary atoms and to add necessary ones at standard bond lengths and bond angles (12). 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 (13). In the MM2 force field, the inclusion of special parameters to account for the lone pairs of electrons on the oxygen atoms 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.

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 find other minimum energy conformers of each molecule after initial optimization, MMP2 Dihedral or Torsional Angle Driver studies were performed (14). We have shown previously that the non-aromatic rings of the cannabinoids can assume more than one conformation and that in certain cases it is a higher energy ring conformer that is more likely the bioactive form (10). Consequently, studies of alternate ring conformations of the cannabinoids are fundamental to the studies described here.

To study the alternate conformation of the carbocyclic ring in **1-3**, the C2-C3-C4-C5 torsional angle was driven. The Torsional Angle Driver study of the carbocyclic ring was an endocyclic drive in which the ring was driven in small increments (1°-10°) over a range of 120°. In each case, if a different ring conformation for a molecule was identified by the driver studies, the geometry of this new conformer was then re-optimized using MMP2(85). After alternate carbocyclic ring conformations were identified, only those conformers which were within 6.00 kcal/mol above the lowest minimum energy conformer (global minimum) were considered accessible (15).

Unlike traditional cannabinoids such as (-)-Δ^9-tetrahydrocannabinol (Δ^9-THC), CBD and its analogs possess an additional point of flexibility. Rotation about the C1'-C3 bond in these compounds allows the cyclohexene ring to rotate relative to the aromatic ring. In order to locate the minimum energy positions of these two rings with respect to one another, as well as to ascertain the rotational energy profile for rotation about the C1'-C3 bond, additional Torsional Angle Driver studies were performed. In these, the exocyclic (C2'-C1'-C3-C2) angle was driven around 360° in 10° increments. Rotational studies of the isopropenyl moiety were also conducted by driving the C3-C4-C8-C10 torsional angle 360° in 10° increments. As it is possible for the cyclohexene ring in CBD and its analogs to exist in more than one conformation, this rotational energy profile was calculated for each minimum energy carbocyclic ring conformer identified above. Rotamers of each minimum energy carbocyclic ring conformer were considered to be accessible if their energies were within 6.00 kcal/mol of the global minimum energy conformer.

Receptor Essential Volume Screen. All minimum energy conformers of CBD and its analogs were screened for their ability to clear the cannabinoid REV Map (10). C1'-C3 rotamers of the global minimum structure of CBD and its analogs were also screened. Rotamers of the other minimum energy carbocyclic ring conformers proved to be too high in energy to consider (i.e. more than 6.00 kcal/mol above the global minimum). The conformers were first superimposed on the REV template molecules by fitting the molecules together at C1',C2',C3' and the phenolic hydroxyl oxygen at C2'. Each accessible conformer of CBD and its analogs was then contoured at its van der Waals' radii and the structure assessed for protrusion into the previously calculated REV region (10).

Synthesis of Desoxycannabidiol. Desoxy-CBD, **3**, was prepared by the condensation of p-mentha-2,8-dien-1-ol with 3-pentylphenol following Petrzilka's method for the synthesis of CBD from olivetol (16). 3-pentylphenol was prepared by the dimetalation of m-cresol with n-BuLi/t-BuOK followed by in situ alkylation with butylbromide. Condensation of 3-pentylphenol with p-mentha-2,8-dien-1-ol mediated by DMF-dineopental acetal afforded predominantly two products. The more polar product was identified as desoxy-CBD by its

NMR spectrum and its facile cyclization ($BF_3 \cdot Et_2O$) and subsequent NMR comparison to the known desoxy-Δ^9-THC (17). The less polar product was identified by NMR as the p-mentha-1,8-dien-3-yl 3-n-pentylphenyl ether. Treatment of the ether with $BF_3 \cdot Et_2O$ also afforded desoxy-Δ^9-THC presumably by cleavage of the phenyl allyl ether, alkylation of the phenol at the 6-position by the allylic cation, and subsequent cyclization. Further details of the synthesis of desoxy-CBD as well as its characterization will be reported elsewhere.

Mouse Vas Deferens Assay. *Drugs.* Desoxy-CBD, **3**, was mixed with 2 parts of Tween 80 by weight and dispersed in a 0.9% aqueous solution of NaCl (saline) as described previously for Δ^9-THC (6).

Tissue Experiments. Vasa deferentia were obtained from albino MF1 mice weighing 50 to 56 g. Each tissue was mounted in a 4 ml organ bath at an initial tension of 0.5 g using the method described in (6). The baths contained Krebs' solution which was kept at 37°C and bubbled with 95% O_2 and 5% CO_2. The composition of the Krebs' solution was (mM); NaCl 118.2, KCl 4.75, KH_2PO_4 1.19, $NaHCO_3$ 25.0, glucose 11.0 and $CaCl_2 \cdot 6H_2O$ 2.54. Isometric contractions were evoked by stimulation with 0.5 s trains of three pulses of 110% maximal voltage (train frequency 0.1 Hz; pulse duration 0.5 ms) through platinum electrodes attached to the upper and lower ends of each bath. Stimuli were generated by a Grass S48 stimulator and contractions were registered on a polygraph recorder (Grass model 7D) using Pye Ether UF1 transducers. Each tissue was subjected to several periods of stimulation, the first of these beginning after the tissue had equilibrated but before drug administration and continuing for 11 min. All other stimulation periods lasted for 5 min. Desoxy-CBD was added after each stimulation period, in progressively increasing doses. Baths were washed out by overflow after each 5 min stimulation period, immediately before drug administration. Drug additions were made in a volume of 10 µl.

Analysis of Data. Values are expressed as means and limits of error as standard errors. Inhibition of the electrically-evoked twitch response is expressed in percentage terms and has been calculated by comparing the amplitude of the twitch response after each addition of desoxy-CBD with its amplitude immediately before the first addition of this drug. The concentration of desoxy-CBD producing a 50% reduction in the amplitude of electrically-evoked contractions (IC_{50} value) was calculated by nonlinear regression analysis using GraphPAD InPlot (GraphPAD Software, San Diego). GraphPAD InPlot was also used to construct a sigmoid log-concentration response curve.

Results

Computational Studies. The carbocyclic ring in CBD was found to possess two conformations which correspond to two different half-chair conformations of this ring, termed here half-chair I and half-chair II. In the global minimum energy conformer of CBD, (half-chair I, C2-C3-C4-C5=45°), the carbocyclic ring is nearly perpendicular to the aromatic ring with a C2'-C1'-C3-C2 value of -130°. The isopropenyl moiety is oriented such that C3-C4-C8-C10 has a value of -78°. Rotational studies of the global minimum conformer revealed that three other minima of half-chair I exist. These correspond to alternate ring, as well as isopropenyl orientations. Table I summarizes information about these conformers.

TABLE I
CBD Conformers

FORM	C2-C3-C4-C5 (degrees)	C2'-C1'-C3-C2 (degrees)	C3-C4-C8-C10 (degrees)	ΔFSE* (kcal/mol)
Half Chair I	45	-130	-78	0.00
	44	-127	104	0.05
	44	50	-79	0.96
	43	50	106	0.85
Half Chair II	-41	-147	152	3.90
	-45	-148	-32	5.38
	-44	28	156	4.19
	-45	29	-32	5.64
	-53	25	43	6.32

* FSE = final steric energy

The lowest energy minimum which corresponds to the alternate half-chair form of CBD (half-chair II, C2-C3-C4-C5 = -41°) was found to be 3.90 kcal/mol above the global minimum. Here also the rings and isopropenyl group could exist in other minimum energy positions. These positions are also summarized in Table I.

REV Assessment. Figure 1a depicts the global minimum conformer of CBD, **1**, with respect to the REV. It is clear from this figure that this conformer of CBD does not clear the REV. When the other conformers of CBD listed in Table I were assessed for their ability to clear the REV, none were found able to clear this region either. However, two rotamers of the global minimum energy form of CBD were found able to clear. These correspond to C2'-C1'-C3-C2 values of -20° and -10°. The -10° rotamer is pictured in Figure 1b with the REV. The energy expense required to clear was quite high, 14.23 kcal/mol for -20° to 12.39 kcal/mol for -10°. This energy expense **exceeds** our 6.00 kcal/mol cut off for accessibility (15). These results indicate that the energy expense for CBD to clear the REV is prohibitive. Consequently, CBD may not be able to adopt the shape required for fitting at the binding site for cannabinoids at the CB1 receptor.

New Analogs. Analogs of CBD, Compounds **2** and **3**, were designed to ascertain if removal of either the isopropenyl group at C4 (Compound **2**) or the C6' hydroxyl (Compound **3**) would result in a lower energy expense to clear the REV. Table II presents information about the minima of these compounds. For Compound **2**, rotamers with C2'-C1'-C3-C2 values of -10° to -30° were able to clear the REV. However, the energy expense increased from 8.75 kcal/mol for the -10° rotamer to 13.29 kcal/mol for the -30° rotamer. Using a 6.00 kcal/mol cut off for accessibility then, **2** cannot clear the REV. For Compound **3**, rotamers with C2'-C1'-C3-C2 values of -60° to 0° were able to clear the REV. However, only the -60° and -50° rotamers were within the 6.00 kcal/mol cut off for accessibility (ΔFSE = 3.64 kcal/mol for the -60° rotamer and 5.51 kcal/mol for the -50° rotamer). Compound **3** possesses all required pharmacophoric requirements for cannabinoid activity (9), as well as the ability to adopt the hypothesized shape required for interaction at the CB1 receptor. Taken together, these results suggest that Compound **3** should be an active compound.

TABLE II
Half-Chair I Conformers of Compounds **2** and **3**

COMPOUND	C2-C3-C4-C5 (degrees)	C2'-C1'-C3-C2 (degrees)	C3-C4-C8-C10 (degrees)	ΔFSE* (kcal/mol)
2	45	-130	N/A	0.00
		50	N/A	0.96
3	47	-131	-78	0.00
		50	-78	0.47

* FSE = final steric energy

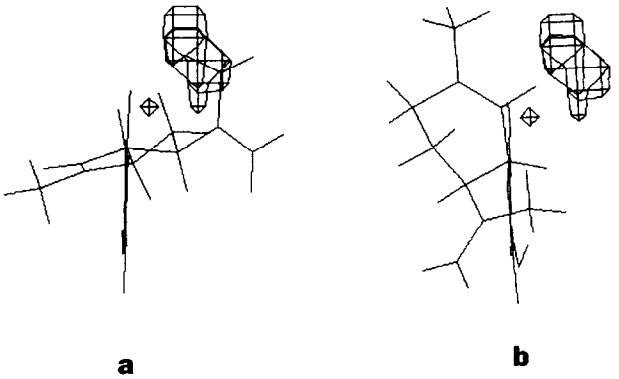

Fig. 1

(a) The global minimum conformer of CBD is shown here in relation to the REV map. (b) The C2'-C1'-C3-C2 = -10° rotamer of the global conformer of CBD is shown here clearing the REV. Each structure is viewed in a direction parallel to the vector from C4' to C6'. The side chain of each has been removed to permit better visualization of the rest of the molecule. Reference 10 provides details of the REV calculation.

Pharmacological Studies. Desoxy-CBD, **3**, was found to produce a concentration related inhibition of electrically-evoked contractions of the vas deferens and to have an IC_{50} of 30.9 nM and a log-concentration response curve that is sigmoid in shape ($r^2 = 0.962$) (Figure 2). It therefore differs markedly from CBD which at concentrations of up to 100 nM has been reported to have no detectable inhibitory effect on electrically-evoked contractions of the mouse vas deferens (5). Desoxy-CBD behaves as a partial agonist in the vas deferens. Thus the gradient of its log-concentration response curve is shallower than that of cannabinoid receptor agonists such as Δ^9-THC or CP-55,940 (8) and the maximal degree of inhibition it can produce is well below 100% (Figure 2).

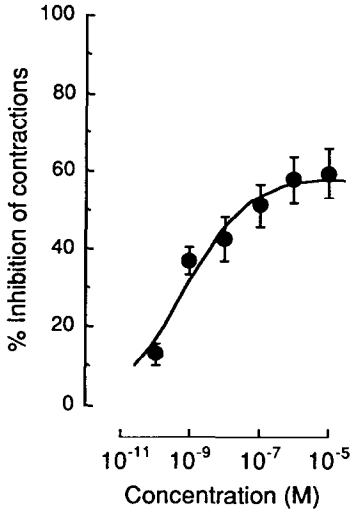

Fig. 2

Mean concentration response curve for desoxy-CBD. Each symbol represents the mean value ± S.E. of inhibition of electrically-evoked contractions of the mouse vas deferens expressed as a percentage of the amplitude of the twitch response measured immediately before addition of desoxy-CBD to the organ bath (n = 8 different vasa deferentia).

Conclusions

The results obtained here for desoxy-CBD, **3**, support the hypothesis that one requirement for cannabinoid CB1 receptor mediated activity is that the molecule be able to adopt a shape such that it avoids a sterically forbidden region located near the top of the carbocyclic ring in the bottom face of the molecule. Pharmacological results suggest that desoxy-CBD is capable of binding to and activating the CB1 receptor, albeit with reduced efficacy. Recently, Tius et al reported the synthesis of two conformationally restricted hybrids of CP-55,940 and (-)-9-nor-9β-hydroxy-hexahydrocannabinol ((-)-9-nor-9β-OH-HHC) in which one of the two C6 methyl groups of (-)-9-nor-9β-OH-HHC (dibenzopyran numbering system) was replaced by a hydroxyethyl moiety (18). When the α-axial C6 methyl was replaced, a compound with greatly reduced activity resulted. Such results seem to point to yet another sterically forbidden region in the bottom face of the molecule (behind C6 of the dihydropyran ring in traditional cannabinoids). Inspection of the rotamers of desoxy-CBD able to clear the REV reveals atoms of the isopropenyl moiety very near this lower region. We are currently studying this lower region to ascertain if its existence can account for the reduced efficacy of desoxy-CBD.

Acknowledgements

This work was supported by NIDA Grant 03934 (to P.H.R.), by a Council on Undergraduate Research AIURP Fellowship (to R.D.B.), and by grants 034924 and 039538 (to R.G.P.) from the Wellcome Trust.

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