Communications to the Editor

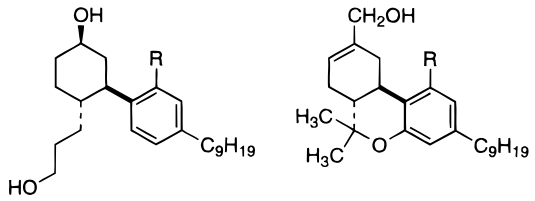
Synthesis and Pharmacology of a Very Potent Cannabinoid Lacking a Phenolic Hydroxyl with High Affinity for the CB2 Receptor

John W. Huffman,*,† Shu Yu,† Vincent Showalter,‡ Mary E. Abood,‡ Jenny L. Wiley,‡ David R. Compton,‡ Billy R. Martin,‡ R. Daniel Bramblett,§ and Patricia H. Reggio§

Department of Chemistry, Clemson University, Clemson, South Carolina 29634-1905, Department of Pharmacology and Toxicology, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298, and Department of Chemistry, Kennesaw State University, 1000 Chastain Road, Kennesaw, Georgia 30144-5591

Received June 3, 1996

Song and Bonner have very recently described a mutant (Lys 192 Ala) CB1 receptor in which the binding of CP 55,940 (**1**), and the very potent cannabinoid HU-210 (the dimethylheptyl analogue of 11-hydroxy-Δ^8-tetrahydrocannabinol, 11-hydroxy-Δ^8-THC-DMH, **2**) is greatly attenuated.[1] These authors conclude that it is likely that in the wild-type receptor Lys 192 is hydrogen bonded to the phenolic hydroxyl of cannabinoids **1** and **2** and that the loss of a terminal amino group when Lys 192 is replaced by alanine is responsible for the decrease in affinity.[1]



1 R = OH **2** R = OH

13 R = H **3** R = H

C_9H_{19} = 1,1-Dimethylheptyl

A computer model of the cannabinoid brain (CB1) receptor[2] was examined for possible ligand interactions (see the Experimental Section included in the Supporting Information for model building details). On the basis of Song and Bonner's mutation studies of Lys 192, 11-hydroxy-Δ^8-THC-DMH (**2**) was docked so that Lys 192 could interact with the phenolic hydroxyl group at C-1. An extensive amount of cannabinoid structure−activity relationship (SAR) data indicate that the cannabinoid C-3 side chain is a key element in CB1 receptor recognition.[3−6] It was found that with the phenolic hydroxyl of **2** interacting with Lys 192, the C-3 side chain can interact with a hydrophobic pocket formed by Val 351 and Ile 354. In this docking position, Tyr 275 at the top of Helix 5 can hydrogen bond to the 11-hydroxyl of **2**, while Val 196 creates steric hindrance behind the carbocyclic ring of 11-hydroxy-Δ^8-THC-DMH. The existence of such a sterically occluded region has been postulated in cannabinoid SAR for some time.[7,8]

Since traditional cannabinoid SAR, mutation studies, and the modeling studies described above indicate that the phenolic hydroxyl is a key interaction site at CB1, it was assumed that a 1-deoxy cannabinoid would show little affinity for the CB1 receptor and little potency *in vivo*.[3,4] The SAR and modeling data suggest that a cannabinoid lacking the phenolic hydroxyl should exhibit poor affinity for the wild-type CB1 receptor similar to the poor affinity observed for the traditional cannabinoids, CP 55,940 (**1**) and 11-hydroxy-Δ^8-THC-DMH (**2**), for binding to the mutant receptor in which a lysine has been replaced by an alanine. A suitable compound to test this hypothesis appeared to be **3**, the 1-deoxy analogue of the very potent cannabinoid 11-hydroxy-Δ^8-THC-DMH.

The synthesis of deoxy cannabinoid **3** was carried out using methodology developed for the synthesis of 1-nor-9-carboxy-Δ^9-tetrahydrocannabinol,[9] and the enantiodivergent synthesis of both enantiomers of nabilone.[10] As shown in Scheme 1, the aromatic component for the synthesis, 2-bromo-5-(1,1-dimethylheptyl)methoxybenzene (**4**),[11] was prepared in four steps from 1,3-dimethoxy-5-(dimethylheptyl)benzene (**5**).[10,12] The aryllithium derived from **4** was added to apoverbenone to give, after oxidative rearrangement, enone **6**.[11] Dissolving metal-reduction of **6** proceeded stereoselectively to provide saturated ketone **7**.[11] Ether cleavage, followed by sequential treatment with $SnCl_4$ and $AlCl_3$, gave deoxynabilone (**8**).[11] Although ketone **6** could be converted to the corresponding enolate, this enolate was unreactive to a variety of reagents designed to provide triflate **9**.[13,14] The triflate was ultimately prepared by the procedure of Stang, employing triflic anhydride in the presence of 2,6-di-*tert*-butylpyridine.[15] Palladium-mediated carbonylation of **9** in the presence of methanol gave ester **10**,[11,16] which provided **3**[11] after reduction.

The affinity of **3** for the CB1 receptor was determined by measuring its ability to displace [^{3}H]CP 55,940 (**1**) from its binding site in a membrane preparation from rat brain as described by Compton *et al.*[17] The affinity for the peripheral (CB2) receptor was determined by measuring the ability of **3** to displace [^{3}H]CP 55,940 from a cloned human receptor preparation using the procedure described by Showalter *et al.*[18] In contrast to expectations, **3** has high affinity for the CB1 receptor (K_i = 1.2 ± 0.1 nM) and even greater affinity for the CB2 receptor (K_i = 0.032 ± 0.19 nM). These data are included in Table 1 along with those for 11-hydroxy-Δ^8-THC-DMH (**2**), Δ^9-tetrahydrocannabinol (Δ^9-THC, **14**), the active constituent of marijuana, and the 1′,1′-dimethylheptyl analogue of Δ^8-tetrahydrocannabinol (Δ^8-THC DMH, **11**). The *in vivo* pharmacology of **3** was evaluated in the mouse model of cannabimimetic activity which consists of measuring spontaneous activity (SA), antinociception (as tail flick, TF), and hypothermia (as rectal temperature, RT).[19] These data are also

* Address correspondence to this author at: Department of Chemistry, Clemson University, Clemson, South Carolina 29634-1905.
† Clemson University.
‡ Medical College of Virginia, Virginia COmmonwealth University.
§ Kennesaw State University.

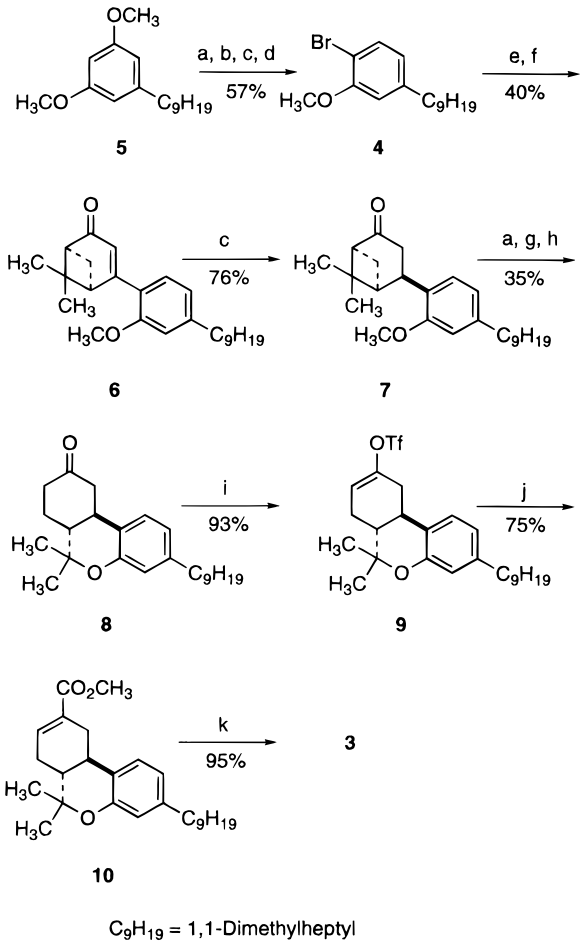
S0022-2623(96)00394-9 CCC: $12.00

Table 1. Pharmacological Responses (Mean ± SEM) of 1-Deoxy-11-hydroxy-Δ^8-THC-DMH (**3**) and Related Compounds

compound	K_i (nM)		ED_{50} (μmol/kg)		
	CB1	CB2	SA	TF	RT
Δ^9-THC (**14**)	41 ± 2[a]	36 ± 10[b]	2.9[c]	4.8[c]	4.5[c]
11-hydroxy-Δ^8-THC-DMH (**2**)	0.73 ± 0.11[a]	0.52 ± 0.05[d]	0.01[e]	0.02[e]	0.05[e]
Δ^8-THC-DMH (**11**)	0.77 ± 0.11[c]	ND	0.27[c]	0.14[c]	0.15[c]
1-deoxy-11-hydroxy-Δ^8-THC-DMH (**3**)	1.2 ± 0.1	0.032 ± 0.019	0.12	0.15	0.39
deoxy-Δ^8-THC-DMH (**12**)	23 ± 7	2.9 ± 1.6	6.4	1.6	3.1

[a] Reference 14. [b] Reference 18. [c] Martin, B. R.; Compton, D. R.; Semus, S.; Lin, S.; Marciniak, C.; Grzybowska, J.; Charalambous, A. Makriyannis, A. *Pharmacol. Biochem. Behav.* **1993**, *46*, 295−301. [d] Felder, C. C.; Joyce, K. E.; Briley, E. M.; Mansouri, J.; Mackie, K.; Olivier, B.; Lai, Y.; Ma, A. L.; Mitchell, R. L. *Mol. Pharmacol.* **1995**, *48*, 443−450. [e] Little, P. J.; Compton, D. R.; Mechoulam, R.; Martin, B. R. *Pharmacol. Biochem. Behav.* **1989**, *32*, 661−666.

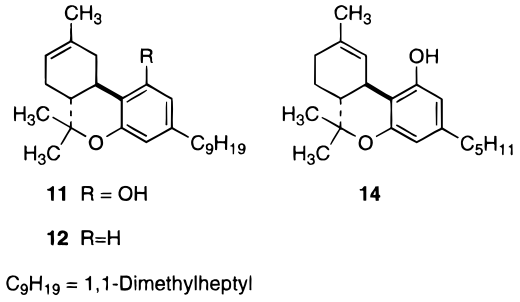
Scheme 1[a]



[a] (a) NaSPr/DMF, 120 °C; (b) $(EtO)_2P(O)H$, Et_3N/CCl_4, 0−25 °C; (c) Li/NH_3, THF, −78 °C; (d) Br_2/HOAc, 0−25 °C; (e) BuLi/THF, 0 °C then apoverbenone; (f) PDC/CH_2Cl_2, 25 °C; (g) $SnCl_4/CH_2Cl_2$, 25 °C; (h) $AlCl_3/CH_2Cl_2$, 25 °C; (i) Tf_2O, 2,6-di-*tert*-butyl-4-methylpyridine/CH_2Cl_2, 40 °C; (j) Et_3N, $Pd(OAc)_2$, Ph_3P, CO, CH_3OH/DMF, 45 °C; (k) $LiAlH_4/Et_2O$, 0−25 °C.

included in Table 1, with those of 11-hydroxy-Δ^8-THC-DMH (**2**) and Δ^9-THC (**14**). 1-Deoxy-11-hydroxy-Δ^8-THC-DMH (**3**) also produced dose dependent cannabimimetic effects in the rat discrimination procedure (ED_{50} = 0.42 mg/kg, 95% CL: 0.12−1.5), an animal model of the subjective effects of cannabis intoxication in humans.[20] The drug discrimination studies were carried out using the procedure described by Wiley *et al.*[21]

As can be seen from the data summarized in Table 1, 1-deoxy-11-hydroxy-Δ^8-THC-DMH (**3**) is a very potent cannabinoid, both *in vitro* and *in vivo* in contrast to expectations based on traditional SAR.[3,4] In order to provide a rationalization for the enhanced potency of **3**, modeling studies have been carried out which indicate



that the side chain of Lys 192 has the length and flexibility to form a hydrogen bond with the C-11 hydroxyl, while the C-3 side chain interacts with the hydrophobic binding pocket.[22] There is a net loss of one hydrogen-bonding interaction upon removal of the phenolic hydroxyl group of 11-hydroxy-Δ^8-THC-DMH, which may account for the decrease in potency of **3** relative to **2**.

On the basis of this model, it appeared probable a cannabinoid lacking both the C-11 and phenolic hydroxyls should show much attenuated potency. Accordingly, 1-deoxy-Δ^8-THC DMH (**12**) was prepared from Δ^8-THC DMH (**11**) by reaction with diethyl chlorophosphate, followed by reduction of the phosphate ester using Li in liquid ammonia. In contrast to expectations, deoxycannabinoid **12** showed high affinity for both the CB1 and CB2 receptors (K_i = 23 ± 7 and 2.9 ± 1.6 nM, respectively). The *in vivo* pharmacology (Table 1) indicated that **12** was approximately equal in potency to Δ^9-THC. Docking studies indicated that the orientation of **12** would have to be inverted relative to that of Δ^9-THC, 11-hydroxy-Δ^8-THC-DMH (**2**), or 1-deoxy-11-hydroxy-Δ^8-THC-DMH (**3**) in order to account for the receptor affinity. In this inverted orientation, the pyran oxygen hydrogen bonds to Lys 192, which increases the distance between the hydrophobic binding pocket and C-3, the atom to which the side chain is attached. Although there is now a longer distance to traverse for interaction with the hydrophobic binding pocket, the dimethylheptyl side chain of **12** can still reach this pocket, albeit with fewer carbon atoms participating in the hydrophobic interaction.

Following the completion of this work, the synthesis of **12** and its binding affinity for the human CB1 and CB2 receptors was reported by Gareau *et al.*[23] The method employed for the preparation of **12** was the same as that outlined above; however, the affinity data differed considerably from those cited above. The reported affinities for the human CB1 and CB2 receptors are K_i = 249.7 ± 31.0 and 20.8 ± 11.2 nM, respectively, both of which are 1 order of magnitude less than that determined in this work. The difference in

the affinity data for CB1 may be due to the fact that Gareau *et al.* employed the human receptor, while our data were obtained using a rat membrane preparation; however, the human and rat receptors are virtually identical (97% homology).[24] Both sets of binding data for CB2 were obtained using the human receptor, and the reported differences may be due to differences in the experimental procedures employed in obtaining the affinity data.

In contrast to the classical generalizations of cannabinoid SAR, which state that for those compounds based on the dibenzopyran skeleton, a phenolic hydroxyl at C-1 is necessary, both 1-deoxy-11-hydroxy-Δ^8-THC-DMH (**3**) and 1-deoxy-Δ^8-THC-DMH (**12**) have significant affinity for both the CB1 and CB2 receptors. Further, both compounds exhibit pharmacological properties in the mouse similar to those of Δ^9-THC. 1-Deoxy-11-hydroxy-Δ^8-THC-DMH (**3**) also shows typical cannabinoid behavior in the rat discrimination procedure. To the best of our knowledge, the only other 1-deoxy cannabinoid which has been evaluated using contemporary methodology is 1-deoxy-CP 55,940 (**13**), which has approximately the same affinity as Δ^9-THC for the CB1 receptor (K_i = 40.2 ± 13.5 nM), but is approximately 200 times less potent than CP 55,940 *in vivo*.[5] On the basis of these data, it is apparent that a phenolic hydroxyl group at C-1 is *not* essential for cannabinoid activity. The modeling studies described above suggest that the structural features necessary for typical cannabinoid activity are the presence of an oxygen atom to which Lys 192 can hydrogen bond, and a lipophilic structural unit which can simultaneously interact with the lipophilic pocket on Helix 6 of the CB1 receptor.

Acknowledgment. The work at Clemson was supported by Grant DA03590, that at Kennesaw State University by Grant DA03934, and that at Virginia Commonwealth University by Grants DA03672 (Billy R. Martin) and DA05274 (Mary E. Abood) and Training Grant DA07027 (Vincent M. Showalter), all from the National Institute on Drug Abuse.

Supporting Information Available: Details of the synthesis of 1-deoxy-HU-210 (**3**) and 1-deoxy-Δ^8-THC-DMH (**12**) and details of the modeling studies (13 pages). Ordering information is given on any current masthead page.

References

(1) Song, Z. H.; Bonner, T. I. A lysine residue of the cannabinoid receptor is critical for receptor recognition by several agonists but not WIN-55,212. *Mol. Pharmacol.* **1996**, *49*, 891−896.
(2) Bramblett, R. D.; Panu, A. M.; Ballesteros, J. A.; Reggio, P. H. Construction of a 3D model of the cannabinoid CB1 receptor: determination of helix ends and helix orientation. *Life Sci.* **1995**, *56*, 1971−1982.
(3) Razdan, R. K. Structure-activity relationships in cannabinoids. *Pharmacol. Rev.* **1986**, *38*, 75−149.
(4) Rapaka, R. S.; Makriyannis, A. *Structure-Activity Relationships of the Cannabinoids*; NIDA Research Monograph 79; National Institute on Drug Abuse: Rockville, MD, 1987.
(5) Melvin, L. S.; Milne, G. M.; Johnson, M. R.; Subramian, B.; Wilken, G. H.; Howlett, A. C. Structure-activity relationships for cannabinoid receptor-binding and analgesic activity: studies of bicyclic cannabinoid analogs. *Mol. Pharmacol.* **1993**, *44*, 1008−1015.
(6) Ryan, W.; Singer, M.; Razdan, R. K.; Compton, D. R.; Martin, B. R. A novel class of potent tetrahydrocannabinols (THCs): 2′-yne-Δ^8-and Δ^9-THCs. *Life Sci.* **1995**, *56*, 2013−2020.
(7) Reggio, P. H.; Panu, A. M.; Miles, S. Characterization of a region of steric interference at the cannabinoid receptor using the active analog approach. *J. Med. Chem.* **1993**, *36*, 1761−1771.
(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) Huffman, J. W.; Zhang, X.; Wu, M.-J.; Joyner, H. H.; Pennington, W. T. Synthesis of (±)-11-nor-9-carboxy-Δ^9-tetrahydrocannabinol: new synthetic approaches to cannabinoids. *J. Org. Chem.* **1991**, *56*, 1481−1489.
(10) Huffman, J. W.; Joyner, H. H.; Lee, M. D.; Jordan, R. D.; Pennington, W. T. Synthesis of both enantiomers of nabilone from a common intermediate. Enantiodivergent synthesis of cannabinoids. *J. Org. Chem.* **1991**, *56*, 2081−2086.
(11) All new compounds were characterized by ^{1}H and ^{13}C NMR and gave either acceptable analytical or HRMS data. Purity was established by TLC and ^{13}C NMR.
(12) Dominianni, S. J.; Ryan, C. W.; De Armitt, C. W. Synthesis of 5-(*tert*-alkyl)resorcinols. *J. Org. Chem.* **1977**, *42*, 344−346.
(13) McMurry, J. E.; Scott, W. J. A method for the regioselective synthesis of enol triflates by enolate trapping. *Tetrahedron Lett.* **1983**, *24*, 979−982.
(14) Comins, D. L.; Dehghani, A. Pyridine derived triflating reagents: an improved preparation of vinyl triflates from metallo enolates. *Tetrahedron Lett.* **1992**, *33*, 6299−6302.
(15) Stang, P. J.; Treptow, W. Single step improved synthesis of primary and other vinyl trifluoromethanesulfonates. *Synthesis* **1980**, 283−284.
(16) Cacchi, S.; Morera, E.; Ortar, G. Palladium catalyzed carbonylation of enol triflates. A novel method for one-carbon homologation of ketones to α,β-unsaturated carboxylic acid derivatives. *Tetrahedron Lett.* **1985**, *26*, 1109−1112.
(17) Compton, D. R.; Rice, K. C.; De Costa, B. R.; Razdan, R. K.; Melvin, L. S.; Johnson, M. R.; Martin, B. R. Cannabinoid structure-activity relationships: correlation of receptor binding and *in vivo* activities. *J. Pharmacol. Exp. Ther.* **1993**, *265*, 218−226.
(18) Showalter, V. M.; Compton, D. R.; Martin, B. R.; Abood, M. E. Evaluation of binding in a transfected cell line expressing a peripheral cannabinoid receptor (CB2): identification of cannabinoid receptor subtype selective ligands. *J. Pharmacol. Exp. Ther.*, in press.
(19) Little, P. J.; Compton, D. R.; Johnson, M. R.; Melvin, L. S.; Martin, B. R. Pharmacology and stereoselectivity of structurally novel cannabinoids in mice. *J. Pharmacol. Exp. Ther.* **1988**, *247*, 1046−1051.
(20) Balster, R. L.; Prescott, W. R. Δ^9-Tetrahydrocannabinol discrimination in rats as a model for cannabis intoxication. *Neurosci. Biobehav. Rev.* **1992**, *16*, 55−62.
(21) Wiley, J. L.; Lowe, J. A.; Balster, R. L.; Martin, B. R. Antagonism of the discriminative stimulus effects of Δ^9-tetrahydrocannabinol in rats and rhesus Monkeys. *J. Pharmacol. Exp. Ther.* **1995**, *275*, 1−6.
(22) An alternative, but energetically less favorable, docking position maintains the hydrogen bond of the C-11 hydroxyl with Tyr 5.39, and the C-3 side chain interacts with the hydrophobic binding pocket.
(23) Gareau, Y.; Dufresne, C.; Gallant, M.; Rochette, C.; Sawyer, N.; Slipetz, D. M.; Tremblay, N.; Weech, P. K.; Metters, K. M.; Labelle, M. Structure activity relationships of tetrahydrocannabinol analogues on human cannabinoid receptors. *BioMed. Chem. Lett.* **1996**, *6*, 189−194.
(24) Gérard, C. M.; Mollereau, C.; Vassart, G.; Parmentier, M. Molecular cloning of a human cannabinoid receptor which is also expressed in testis. *Biochem. J.* **1991**, *279*, 129−134.

JM960394Y