Cannabinol Derivatives: Binding to Cannabinoid Receptors and Inhibition of Adenylylcyclase

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Several derivatives of cannabinol and the 1,1-dimethylheptyl homolog (DMH) of cannabinol were prepared and assayed for binding to the brain and the peripheral cannabinoid receptors (CB_1 and CB_2), as well as for activation of CB_1- and CB_2-mediated inhibition of adenylylcyclase. The DMH derivatives were much more potent than the pentyl (i.e., cannabinol) derivatives. 11-Hydroxycannabinol (**4a**) was found to bind potently to both CB_1 and CB_2 (K_i values of 38.0 ± 7.2 and 26.6 ± 5.5 nM, respectively) and to inhibit CB_1-mediated adenylylcyclase with an EC_{50} of 58.1 ± 6.2 nM but to cause only 20% inhibition of CB_2-mediated adenylylcyclase at 10 μM. It behaves as a specific, though not potent, CB_2 antagonist. 11-Hydroxycannabinol-DMH (**4b**) is a very potent agonist for both CB_1 and CB_2 (K_i values of 100 ± 50 and 200 ± 40 pM; EC_{50} of adenylylcyclase inhibition 56.2 ± 4.2 and 207.5 ± 27.8 pM, respectively).

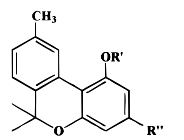
Introduction

Two cannabinoid receptors have been described to date. Both are proteins with seven transmembrane-spanning domains. These receptors were originally found in rat brain and spleen, respectively, and are generally known as the central cannabinoid receptor, CB_1, and the peripheral cannabinoid receptor, CB_2.[1,2] CB_1 is mainly expressed in the central nervous system (CNS). It is also found, to a lesser extent, outside the CNS, in numerous other tissues such as vas deferens, adrenal gland, heart, lung, prostate, uterus, ovary, testis, bone marrow, thymus, and tonsils.[3,4] The CB_2 gene is not expressed in the brain, being found mostly in the immune system. In certain tissues, such as spleen, the mRNA content of CB_2 is particularly high. In blood cell subpopulations, it is found particularly in B-cells.[3,4] It has been suggested that “cannabinoids may exert specific receptor-mediated action on the immune system through the CB_2 receptor”.[4]

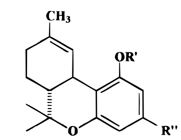
While a considerable amount of work has been done on structure−activity relationships (SAR) as regards binding to CB_1,[5−7] very little is known on binding to CB_2.[8] In the original paper on the identification of CB_2, Munro et al.[9] showed that cannabinol (CBN) (**1a**), which only binds feebly to CB_1, much less so than Δ^9-tetrahydrocannabinol (Δ^9-THC) (**2a**), binds to CB_2 at the same level of potency as Δ^9-THC. The low binding to CB_1 is compatible with the low THC-type *in vivo* activity recorded for CBN when compared to Δ^9-THC.[10] The binding of CBN to CB_2, with a potency equivalent to that of Δ^9-THC, suggests that cannabinol derivatives could serve as a novel starting point for SAR analysis in the cannabinoid series and could possibly lead to selectivity as regards binding to the two cannabinoid receptors.

We present here data on several cannabinol derivatives which have been tested for binding to CB_1 and CB_2.

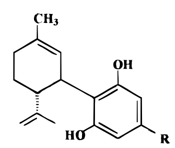
Chart 1



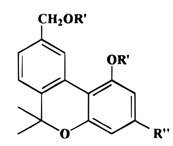
1a, Cannabinol (CBN)
R' = H, R'' = C_5H_{11}
1b, R' = H, R'' = $C(CH_3)_2C_6H_{13}$
1c, R' = $COCH_3$, R'' = $C(CH_3)_2C_6H_{13}$



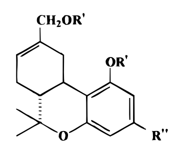
2a, Δ^9-THC, R' = H, R'' = C_5H_{11}
2b, R' = $COCH_3$, R'' = C_5H_{11}
2c, R' = H, R'' = $C(CH_3)_2C_6H_{13}$
2d, R' = $COCH_3$, R'' = $C(CH_3)_2C_6H_{13}$



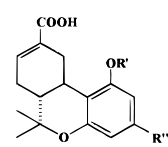
3a, Cannabidiol, R = C_5H_{11}
3b, R = $C(CH_3)_2C_6H_{13}$



4a, R' = H, R'' = C_5H_{11}
4b, R' = H, R'' = $C(CH_3)_2C_6H_{13}$
4c, R' = $COCH_3$, R'' = $C(CH_3)_2C_6H_{13}$



5a, R' = H, R'' = $C(CH_3)_2C_6H_{13}$
5b, R' = $COCH_3$, R'' = $C(CH_3)_2C_6H_{13}$
5c, R' = H, R'' = C_5H_{11}



6a, R' = H, R'' = $C(CH_3)_2C_6H_{13}$
6b, R' = $COCH_3$, R'' = $C(CH_3)_2C_6H_{13}$
6c, R' = H, R'' = C_5H_{11}
6d, R' = $COCH_3$, R'' = C_5H_{11}

Both CB_1 and CB_2 binding assays were performed with transfected cells.[11−13] For comparison purposes, the derivatives were also assayed for CB_1 binding to membranes, as previously described.[14]

Various laboratories, including our own, have established that the signal transduction pathway utilized by the two cannabinoid receptors proceeds through pertussis toxin-sensitive G proteins and inhibition of adenylylcyclase activity.[2] We recently showed that Δ^9-THC binds to both receptors with similar affinity; however, in contrast to its capacity to serve as an agonist for the CB_1 receptor and to inhibit CB_1-mediated adenylylcyclase, Δ^9-THC was only able to induce a very slight inhibition of adenylylcyclase at the CB_2 receptor.[12]

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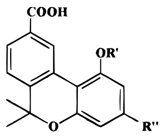
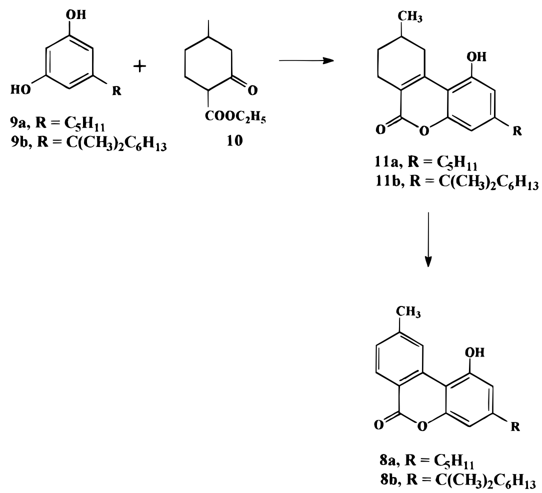
Scheme 1



7a, R' = H, R'' = C_5H_{11}
7b, R' = $COCH_3$, R'' = C_5H_{11}
7c, R' = H, R'' = $C(CH_3)_2C_6H_{13}$
7d, R' = $COCH_3$, R'' = $C(CH_3)_2C_6H_{13}$



Morever Δ^9-THC antagonizes the agonist-induced inhibition of adenylylcyclase mediated by CB_2. Therefore, we concluded that Δ^9-THC constitutes a weak antagonist for the CB_2 receptor.[12] It was thus of interest to find out whether CBN derivatives could selectively activate one of the cannabinoid receptors. Therefore, all compounds were tested for CB_1- as well as CB_2-mediated inhibition of adenylylcyclase. The present paper is apparently the first one which examines the SAR of cannabinol derivatives for binding to the cannabinoid receptors as well as for inhibition of adenylylcyclase.

Chemistry

Cannabinol (**1a**), Δ^9-THC (**2a**), and cannabidiol (**3a**) were extracted from hashish.[15,16] The 1,1-dimethylheptyl (DMH) homolog (**2c**) (at position 3) of Δ^9-THC was prepared by Lewis acid-catalyzed ring cyclization of the DMH homolog of cannabidiol (**3b**),[17] as previously described for the parallel conversion of cannabidiol (**3a**) into Δ^9-THC (**2a**).[16] The DMH homolog of cannabinol (**1b**) was prepared by dehydrogenation with sulfur at 240 °C of **2d** (synthesized by acetylation of **2c**), leading to **1c**, followed by removal of the acetate group with ethanolic sodium hydroxide. The isomeric 1,2-dimethylheptyl homolog of CBN has previously been prepared and shown to be a potent cannabimimetic.[18] 11-Hydroxycannabinol (**4a**) was prepared, as previously described, by selenium dioxide oxidation and dehydrogenation of Δ^9-THC acetate (**2b**) followed by removal of the ester grouping.[19] 11-Hydroxy-Δ^8-THC (**5c**) and its DMH homolog (**5a**) were prepared as previously described.[19,20] Dehydrogenation of the DMH homolog of 11-hydroxy-Δ^8-THC diacetate (**5b**)[20] with sulfur at 240 °C led to the DMH homolog of 11-hydroxycannabinol diacetate (**4c**), which was converted to the diol **4b**.

The same procedure was followed for the conversion of Δ^8-THC-7-oic acid (**6c**)[21] into cannabinol-7-oic acid (**7a**)[22] and of the DMH homolog of (−)-Δ^8-THC-11-oic acid (**6a**)[23] into the DMH homolog of cannabinol-11-oic acid (**7c**). The lactones **8a**,**b** were prepared following a route reported by the groups of Adams and Todd about 50 years ago.[24] Von Pechmann condensation of either olivetol (**9a**) or 3-(1,1-dimethylheptyl)resorcinol (**9b**) with ethyl 5-methyl-1-oxocyclohexane-2-carboxylate (**10**) led to the known tetrahydrodibenzopyrone **11a** or **11b**, respectively.[24] Dehydrogenation of **11a** or **11b** as described above gave **8a**[24] or **8b**, respectively.

Biological Results

All compounds were tested for binding to CB_1 using African green monkey kidney (COS-7) cells transfected with the cDNA of rat CB_1[12,25] as well as rat brain synaptosomal membrane preparations.[14,26] Binding to CB_2 was performed using transfected COS-7 and Chinese hamster ovary (CHO) cells transfected with the cDNA of human CB_2.[9,11,12] The K_i values were determined by displacement of [^{3}H]HU-243, a probe for both cannabinoid receptors.[26,27] For consistency, the results discussed below are those from transfected cells except when specifically indicated. All compounds were also assayed for inhibition of CB_1- and CB_2-mediated adenylylcyclase.[11,12] The results are summarized in Table 1.

First, CBN (**1a**) was compared to Δ^9-THC (**2a**). Under our experimental conditions, CBN was about 2−4 times less potent on binding to either CB_1 or CB_2, in contrast to the data reported by Munro et al.,[9] who found that CBN and Δ^9-THC are equipotent on CB_2. These differences in recorded potencies are not unexpected, as binding data are very sensitive to experimental conditions. The CB_1-mediated adenylylcyclase inhibition data seem to be closer to *in vivo* observations, where, as mentioned above, Δ^9-THC is considerably more potent than CBN. We found that CBN is about 13 times less potent than Δ^9-THC. With CB_2 we see a different profile. While the EC_{50} of CBN inhibition of CB_2-mediated cyclase is 261.2 ± 46.4 nM, Δ^9-THC causes only 21% inhibition at 1 μM. We have previously shown that Δ^9-THC is a weak agonist for CB_2 and can actually antagonize the CB_2-mediated inhibition of adenylylcyclase caused by more potent agonists.[12]

The next derivative to be examined was the DMH homolog of CBN (**1b**). We observed essentially no difference between its binding affinity to CB_1 or CB_2. On both receptors, **1b** was about 100 times more potent than CBN. It was also 100 times more potent than CBN when tested for binding to rat brain membranes. This compound also potently inhibited adenylylcyclase *via* both CB_1 and CB_2: the K_i of inhibition by **1b** of CB_1-mediated cyclase was ca. 660-fold lower, and the CB_2-mediated cyclase K_i was ca. 300-fold lower than that of CBN. This result demonstrates that the exchange of the pentyl group with the DMH group (at position 3) dramatically increases the affinity of the ligand to both CB_1 and CB_2 and makes it a much more potent agonist. A similar result was obtained with other cannabinol derivatives (see below). Neither CBN (**1a**) nor its DMH homolog **1b** express any particular selectivity with regard to the inhibition of adenylyl cylase *via* the two receptors. However, a major metabolite of CBN, namely, 11-hydroxy-CBN (**4a**),[19,22,28] had a different profile. It binds to both receptors with K_i values ca. 5-fold lower than the K_i values recorded for CBN. However, while

Table 1. Binding of Various Cannabinoids to Cannabinoid Receptors and Inhibition of Adenylylcyclase[a,b]

compound	brain synaptosomal binding, CB_1 (K_i, nM)	COS CB_1 and CB_2 binding (K_i, nM)		COS CB_1 and CB_2 adenylylcyclase (EC_{50}, nM)	
		CB_1	CB_2	CB_1	CB_2
CBN (**1a**)	392.2 ± 53.5	211.2 ± 35.0	126.4 ± 26.0	120.0 ± 32.1	261.2 ± 46.4
CBN-DMH (**1b**)	3.3 ± 0.2	2.0 ± 0.3	1.5 ± 0.5	0.18 ± 0.03	0.79 ± 0.021
Δ^9-THC (**2a**)	66.5 ± 5.8	80.3 ± 22.2	32.2 ± 6.7	11.0 ± 2.1	21% inhib at 1 μM
Δ^9-THC-DMH (**2c**)	2.6 ± 0.3	0.241 ± 0.05	0.199 ± 0.05	0.58 ± 0.12	1.01 ± 0.15
11-OH-CBN (**4a**)	188.9 ± 38.3	38.0 ± 7.2	26.6 ± 5.5	58.1 ± 6.2	20% inhib at 10 μM
11-OH-CBN-DMH (**4b**)	1.8 ± 0.3	0.1 ± 0.05	0.2 ± 0.04	0.056 ± 0.004	0.208 ± 0.028
11-OH-Δ^8-THC (**5c**)	33.4 ± 3.8	25.8 ± 2.9	7.4 ± 2.5	39.9 ± 8.9	19% inhib at 1 μM
11-OH-Δ^8-THC-DMH (**5a**)	0.19 ± 0.01	0.1 ± 0.02	0.17 ± 0.01	0.035 ± 0.005	0.078 ± 0.009
Δ^8-THC-11-oic acid-DMH (**6a**)	480.6 ± 40.8	32.3 ± 3.7	170.5 ± 7.8	927.0 ± 39.6	116.2 ± 74.7
Δ^8-THC-11-oic acid-DMH acetate (**6b**)	>10000	11% inhib at 1 μM	9% inhib at 1 μM	no inhib at 1 μM	27% inhib at 1 μM
CBN-11-oic acid (**7a**)	602.4 ± 15.8	<2% inhib at 1 μM	23% inhib at 1 μM	10% inhib at 1 μM	6% inhib at 1 μM
CBN-11-oic acid acetate (**7b**)	>10000	35% inhib at 1 μM	19% inhib at 1 μM	3% inhib at 1 μM	2% inhib at 1 μM
CBN-DMH-11-oic acid (**7c**)	6.1 ± 0.7	6.1 ± 1.1	4.8 ± 1.9	2.4 ± 0.2	5.4 ± 0.3
CBN-DMH-11-oic acid acetate (**7d**)	556.1 ± 40.6	49.8 ± 11.5	17.3 ± 7.4	13.3 ± 3.4	39.3 ± 6.1
lactone **8a**	>10000	14% inhib at 1 μM	14% inhib at 1 μM	7% inhib at 1 μM	no inhib at 1 μM
lactone **8b**	72.5 ± 3.6	32.7 ± 4.9	17.3 ± 5.6	9.6 ± 0.6	4.6 ± 0.3

[a] For details of procedures, see Experimental Section. [b] For structures, see text.

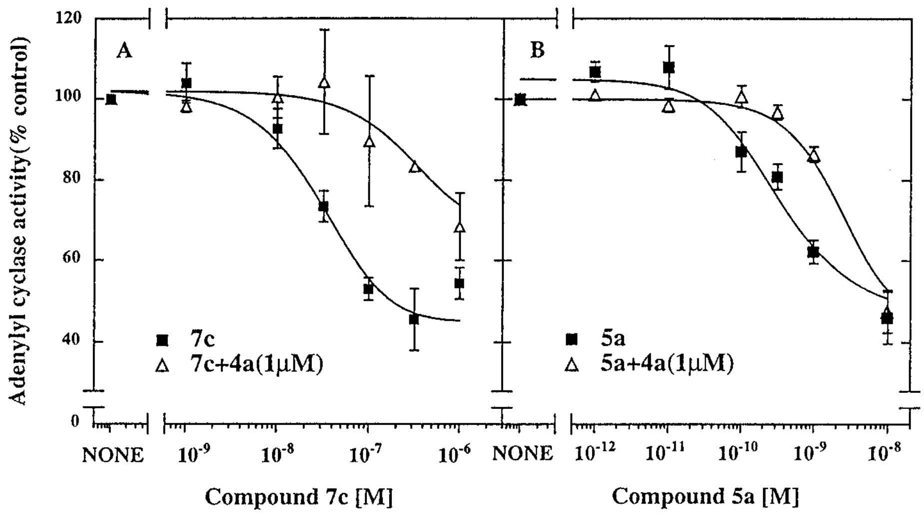


Figure 1. Compound **4a** antagonizes the capacity of the cannabinoid agonists **7c** (A) and **5a** (B) to inhibit the forskolin-stimulated adenylylcyclase activity in CHO cells stably transfected with cDNA of human CB_2. Compounds **4a**, **7c,** and **5a** were added at the indicated concentrations; 100% represents the amount of cAMP in the absence of cannabinoids. Each point is the mean ± standard deviation of three experiments performed in triplicate.

the EC_{50} for the CB_1-mediated inhibition of cyclase was 58.1 ± 6.2 nM, the inhibition of the CB_2-mediated cyclase was negligible: 20% inhibition at 10 μM. This result indicates that **4a** could serve as a functional antagonist of CB_2 as it binds, but does not activate, CB_2. Indeed, it reduces the CB_2-mediated adenylylcyclase inhibitory activity of the potent agonists **5a** (HU-210)[5,8b,20] and **7c** (Figure 1). However while the adenylylcyclase inhibition by **7c** is very strongly reduced by **4a**, that of **5a** is considerably less. This difference may well be due to the higher inhibitory potency of **5a**, compared to **7c**. It should be pointed out that **4a**, a major metabolite of CBN, in its differential activity between CB_1 and CB_2, resembles Δ^9-THC rather than CBN (see above):[12] both Δ^9-THC and **4a** bind to CB_1 and CB_2 and inhibit CB_1-mediated cyclase in the nanomolar range but cause only negligible inhibition of CB_2-mediated cyclase up to micromolar concentrations.

The DMH homolog of 11-hydroxycannabinol (**4b**) is the most potent cannabinoid in the present series. It binds to CB_1 and CB_2 with K_i values of 100 ± 50 and 200 ± 40 pM, respectively. Compound **4b** also inhibits adenylylcyclase *via* both CB_1 and CB_2, at very low concentrations: 56.2 ± 4.2 and 207.5 ± 27.8 pM, respectively. Both the binding constants and EC_{50} values for CB_1 and CB_2 are comparable to the values observed for HU-210 (**5a**).

Cannabinol-11-oic acid (**7a**),[22] as well as its acetate (**7b**), were essentially inactive on binding to either CB_1 or CB_2, as well as in CB_1- or CB_2-mediated cyclase inhibition. However, the DMH homologs, compounds **7c**,**d**, bind to both CB_1 and CB_2 and inhibit adenylylcyclase *via* the two receptors with high potency. The nonacetylated derivative **7c** binds with K_i values of 6.1 ± 1.1 and 4.8 ± 1.9 nM to CB_1 and CB_2, respectively, and inhibits cyclase CB_1- and CB_2-mediated activity with EC_{50} values of 2.4 ± 0.2 and 5.4 ± 0.3 nM, respectively. These results were unexpected as THC-type 11-oic cannabinoid acids (including DMH homologs) such as **6a**,**c** have not been reported to cause psychotropic effects,[10,21,23] which are presumably mediated by CB_1. The acetate **7d** is also active in all these assays, although its activity is 4−7 times lower than the nonacetylated acid (see Table 1). This difference was even larger (90 times) when the acetate **7d** was compared to the nonacetylated acid **7c** with regards to binding to CB_1 on synaptosomal membranes. It is not clear whether the weaker activity of the acetate is due to its own lower intrinsic activity or to a certain amount of nonacetylated **7c** formed by hydrolysis during the assays.

In view of these results (see Discussion), we looked again into the activity of the DMH homolog of Δ^8-THC-7-oic acid (**6a**).[23] This compound, synthesized in our laboratory several years ago, was found then to reduce paw edema and leukocyte adhesion to culture dishes and was considered to be antiinflammatory. It did not cause catalepsy in mice at doses up to 1 mg/kg.[23] We now find that **6a** binds to CB_1 with a rather low K_i of 32.3 ± 3.7 nM but is only a relatively weak inhibitor of CB_1-mediated cyclase (EC_{50} = 927.0 ± 39.6 nM). It binds to the CB_2 receptor with a K_i of 170.5 ± 7.8 nM and inhibits CB_2-mediated cyclase (EC_{50} = 116.2 ± 74.7 nM). This material thus affects signaling *via* CB_2 more effectively than *via* CB_1.

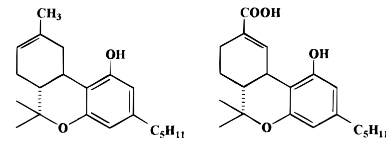
The last series to be examined were cannabinol derivatives in which the pyran ring was modified; instead of the two methyl groups on ring B, a ketone was introduced, thus forming a lactone. Compound **8a** (with a pentyl side chain)[24] did not bind to either CB_1 or CB_2 and did not inhibit adenylylcyclase, while the DMH derivative **8b** was highly potent, binding to both CB_1 and CB_2 with K_i values of 32.7 ± 4.9 and 17.3 ± 5.6 nM, respectively. It inhibited both CB_1- and CB_2-mediated adenylylcyclase with EC_{50} values of 9.6 ± 0.6 and 4.6 ± 0.3 nM, respectively.

Discussion

For reasons discussed in the Introduction, we assumed that CBN derivatives may show differential binding for CB_1 compared to CB_2. The results obtained did not fulfill these expectations. Compounds binding poorly to CB_1 (such as **7a**, **8a**) had the same profile with CB_2, and this was the case also for the more potent compounds (such as **1b, 4a,b, 7c,d, 8b**). However, our results proved to be of interest in another direction: we found that binding to CB_2 (see compound **4a**) does not necessarily imply a parallel level of inhibition of CB_2-mediated adenylylcyclase. As mentioned above, the major CBN metabolite, 11-hydroxy-CBN (**4a**), binds well to both CB_1 and CB_2 (K_i = 38 ± 7.2 and 26.6 ± 5.5 nM, respectively). However, while **4a** inhibits adenylylcyclase strongly *via* CB_1 (EC_{50} = 58.1 ± 6.2 nM), its inhibition of adenylylcyclase *via* CB_2 is negligible (Table 1). Further experiments (see Biological Results and Figure 1) indeed showed that **4a** is a weak antagonist to CB_2. Hence, this CBN metabolite may represent a useful target for future research and possibly a tool in CB_2 investigations. As **4a** is formed *in vivo* from CBN, its presence in the body may have physiological consequences associated with CB_2-promoted activities, possibly in the immune system.

The replacement of the pentyl group at position 3 with the DMH group increased affinity to CB_1 as well as to CB_2 in all compounds tested. The dramatic increase of pharmacological activity associated with such a structural change was first noted about 50 years ago.[24c,d] As indicated above, the DMH homolog of 11-hydroxy-CBN (**4b**) is the most active compound in the present series as regards binding to CB_1 and CB_2, as well as inhibition of adenylylcyclase (see Table 1). It may serve alongside HU-210 (**5a**) as a potent tool in cannabinoid research. Indeed, **4b** shows binding values and cyclase inhibition levels very similar to those of HU-210 (**5a**) (see Table 1).

Oxidation of the 11-CH_3 group in Δ^9-THC (**2a**) or Δ^8-THC (**12**) to a carboxyl group forming THC-7-oic acids (**13** or **6c**, respectively) leads to inactivation, as seen in behavioral assays.[10,21] Indeed, this route is the major



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inactivation pathway of THC metabolism. The DMH homolog of Δ^8-THC, 11-oic acid **6a**, has also been reported to lack THC-type activity,[23] although the reported test range was limited. Unexpectedly, we now find that although **6a** binds to both CB_1 and CB_2 in the 30−170 nM range, the EC_{50} of adenylylcyclase inhibition *via* CB_1 is in the range of 927 ± 39.6 nM. The CB_2-mediated adenylylcyclase is in the intermediate range (116.2 ± 74.7 nM), i.e., a certain separation between CB_1- and CB_2-mediated activation is noted. In the CBN-11-oic acid series, we observed a different profile: while in the pentyl series (**7a,b**), as expected, we recorded no binding or inhibition of cyclase, with DMH-CBN-11-oic acid (**7c**), we found potent binding to both CB_1 and CB_2 and cyclase inhibition (see Table 1). Compounds **6a** and **7c** differ in the conformation of ring A: a planar aromatic ring in **7c** *versus* a half-chair one in **6a**. This conformational difference may represent the molecular basis for the different activities of the two cannabinoids.

A comparison of the binding values of **6a** with those of its CB_1- and CB_2-mediated cyclase inhibition (see Table 1) may explain some of the previously reported properties of **6a**. The relatively high levels of **6a** needed to inhibit adenylylcyclase *via* CB_1 may explain the absence of catalepsy (within the limited dose range tested), in spite of its considerable binding potency, while the ca. 10 times lower levels needed to inhibit adenylyl cylcase *via* CB_2 may be the basis of its reported antiinflammatory activity. These observations may serve to open new leads toward the development of antiinflammatory agents in which wider separations of activity can be achieved than these reported now.

As mentioned above, all compounds presented in Table 1 were tested for binding both in transfected cells and in rat brain synaptosomal preparations. While the general potency trend is comparable in the two assays, some individual differences are striking. Compounds **6b, 7b**, and **8a** are inactive in both assays; **7a** is inactive in transfected cells and poorly active in the membrane assay. The highly potent **1b, 5a**, and **7c** have the same profile in both assays. However 11-hydroxy CBN-DMH (**4b**) has a K_i of 100.0 ± 50.0 pM in transfected cells and nearly 20 times higher K_i (i.e., lower potency) in brain membranes. In the compounds within the intermediate range, the K_i values differ widely: from about 2 times (i.e., essentially equipotent) in **1a, 2a,** and **8b** to about 10 times or more in **7d** and **6a**. The reasons for these differences are not clear, but they should be taken into account when binding data in the cannabinoid series are compared.

In summary, a comparison of the binding potency of several cannabinol derivatives to CB_1 and CB_2 with their capacity to inhibit adenylylcyclase has led to the discovery of a CBN metabolite (11-hydroxy-CBN, **4a**) as a specific, though not potent, CB_2 antagonist and to a new very potent agonist for both CB_1 and CB_2 (11-hydroxy-CBN-DMH, **4b**).

Experimental Section

Chemistry. ^{1}H NMR spectra were measured on a Varian VXR-300S spectrophotometer using TMS as the internal standard. All chemical shifts are reported in ppm. Specific rotations were detected with a Perkin-Elmer 141 polarimeter. Melting points (uncorrected) were determined on a Buchi 530 apparatus. Column chromatography was performed with ICN silica gel 60A. Organic solutions were dried over anhydrous magnesium sulfate. Elemental analyses were obtained for all new compounds (or their acetates) and were ±0.4% of the theoretical values. The analyses were performed at the Elemental Analysis Laboratory of the Hebrew University.

Δ^9-Tetrahydrocannabinol-DMH (2c). Boron trifluoride etherate (5.5 mL) was added to cannabidiol-DMH (**3b**)[17] (5.7 g, 15.4 mmol) in dry dichloromethane (150 mL) containing magnesium sulfate (1 g), under a nitrogen atmosphere. The reaction mixture was stirred at room temperature for 20 min. A saturated solution of sodium bicarbonate was added until the red color observed during the reaction faded. The reaction mixture was washed with water, separated, dried, and evaporated. The oil obtained was chromatographed on a silica gel column. Δ^9-Tetrahydrocannabinol-DMH (**2c**) (3 g, 53%) was eluted with 4% ether in petroleum ether: ^{1}H NMR ($CDCl_3$) δ 6.38, 6.26 (s, 2H), 6.3 (s 1H), 3.16 (d, 1H, J = 11.1 Hz), 2.14 (2H), 0.8−1.8 (m); IR (neat) 3300, 2950, 2850, 1620, 1570 cm^{-1}. Anal. ($C_{25}H_{38}O_2$) C,H.

Δ^9-THC-DMH (**2c**) is not stable. At room temperature it rapidly becomes violet; on TLC after 0.5 h, numerous new spots are observed.

Δ^9-Tetrahydrocannabinol-DMH acetate (2d): ^{1}H NMR ($CDCl_3$) δ 6.67, 6.50 (s, 2H), 6.0 (s, 1H), 3.2 (d, 1H), 2.28 (s, 3H), 2.14 (2H), 1.9−0.8 (m); IR (neat) 2900, 1760, 1620, 1560 cm^{-1}.

Dehydrogenations of 2d, 5b, 6b,d, and 11b. The dehydrogenations were carried out by heating each compound with sulfur at 238−240 °C, under a nitrogen atmosphere, for ca. 4 h. Each mixture was extracted with ether and evaporated. The residue was chromatographed on a silica gel column using variable concentrations of ether in petroleum ether as eluent. Compound **2d** led to cannabinol-DMH acetate (**1c**); compound **5b** gave **4c**; compound **6b** gave **7d**; compound **11b** gave **8b**. For yields and spectroscopic data, see below.

Cannabinol-DMH acetate (1c): obtained in 24% yield, mp 84−86 °C; ^{1}H NMR ($CDCl_3$) δ 7.8, 7.25, 7.13 (s, 3H), 6.85 (d, 1H, J = 2.1 Hz), 6.67 (d, 1H, J = 2.1 Hz), 2.36 (s, 3H), 2.32 (s, 3H), 1.6−0.8 (m); IR (neat) 2930, 1780, 1620, 1560 cm^{-1}. Anal. ($C_{27}H_{36}O_3$) C,H.

11-Hydroxycannabinol-DMH acetate (4c): obtained in 44% yield from **5b**; ^{1}H NMR ($CDCl_3$) δ 8.00, 7.25 (s, 3H) 6.85 (d, 1H, J = 1.5 Hz), 6.68 (d, 1H, J = 1.5 Hz), 5.10 (s, 2H), 2.34 (s, 3H), 2.10 (s, 3H), 1.6−0.85 (m); IR (neat) 2930, 1780, 1740, 1620, 1540 cm^{-1}.

Cannabinol-11-oic acid acetate (7b): obtained in 22% yield from **6d**, mp 149−151 °C; ^{1}H NMR ($CDCl_3$) δ 8.81 (s, 1H), 8.02 (d, 1H, J = 8.1 Hz), 7.38 (d, 1H, J = 8.4 Hz), 6.78, 6.64 (s, 2H), 2.42 (s, 3H), 1.7−0.8 (m); IR (neat) 2960, 1760, 1670, 1610, 1570 cm^{-1}. Anal. ($C_{23}H_{26}O_5$) C, H.

Cannabinol-11-oic acid-DMH acetate (7d): obtained in 20% yield from **6b**; ^{1}H NMR ($CDCl_3$) δ 8.8 (s, 1H), 8.02 (d, 1H, J = 8.1 Hz), 7.38 (d, 1H, J = 8.4 Hz), 6.86, 6.75 (s, 2H), 2.42 (s, 3H), 1.7−0.8 (m); IR (neat) 2960, 1780, 1680, 1620, 1570 cm^{-1}. Anal ($C_{27}H_{34}O_5$) C,H.

Cannabinol-DMH (1b): Hydrolysis of **1c** in ethanolic sodium hydroxide solution gave compound **1b**, mp 95−98 °C; ^{1}H NMR ($CDCl_3$) δ 8.11, 7.22, 7.08, 6.36, 6.4 (s, 5H), 2.33 (s, 3H), 1.5−0.7 (m).

11-Hydroxycannabinol-DMH (4b): Compound **4c** (526 mg, 1.13 mmol) in dry ether (10 mL) was added to lithium aluminum hydride (123 mg) in dry ether (8 mL). The mixture was boiled under reflux for 2 h. The oil obtained after workup was chromatographed on a silica gel column (60 g). Elution with 20% ether in petroleum ether gave, after crystallization from pentane, compound **4b** (340 mg, 78%): mp 128−130 °C; ^{1}H NMR ($CDCl_3$) δ 8.45, 7.255, 7.253, 6.60, 6.40 (s, 5H), 4.75 (s, 2H), 1.65−0.8 (m, 2H). Anal. ($C_{25}H_{34}O_3$) C,H.

Cannabinol-11-oic acid-DMH (7c). Compound **7d** (60 mg) was disolved in 0.6 mL of ethanol. A solution of 60 mg of sodium hydroxide in 0.4 mL of water was added under nitrogen. The solution was stirred at room temperature for 30 min. The solution was acidified with 10% HCl (5 mL); the mixture was extracted with ether, dried with $MgSO_4$, and evaporated. The material obtained was separated on a preparative TLC plate (elution with ether:petroleum ether, 7:3), leading to 41 mg of **7c**, 76% yield: ^{1}H NMR 9.2 (s, 1H), 8.0 (d, 1H), 7.38 (d, 1H), 6.61 (s, 1H), 6.45 (s, 1H), 1.7−0.8 (m).

Cannabinol-11-oic acid (7a): 7b was hydrolyzed in ethanolic sodium hydroxide solution as described for compound **7c** to yield **7a**, 82% yield; ^{1}H NMR δ 9.2 (s, 1H), 8.0 (d, 1H), 7.38 (d, 1H), 6.62 (s, 1H), 6.47 (s, 1H), 1.8−0.8 (m).

Compound 11b. A solution of 3-DMH-resorcinol (**9b**) (3.9 g, 16.5 mmol), ethyl 4-methyl-2-oxocyclohexanecarboxylate (**10**) (4 g, 21.7 mmol), and $POCl_3$ (3.06 mL) in dry benzene (15 mL) was boiled under reflux, under a nitrogen atmosphere, for 3 h. The solution was washed with $NaHCO_3$ followed by water. After drying and evaporation the oil was chromatographed on a silica gel column. Compound **11b**[24c] (40%) was eluted with 10% ether in petroleum ether: mp 158−160 °C (from pentane); ^{1}H NMR ($CDCl_3$) δ 6.83, 6.63 (s, 2H), 3.4 (dd, 2H), 2.8 (dd, 2H), 2.7−0.8 (m); IR (Nujol) 1680, 1610sh, 1580 cm^{-1}; MS M$^+$ 356.

Compound 8b: dehydrogenation of **11b** with sulfur as described above gave compound **8b** (72%), mp 184−185 °C (from pentane); ^{1}H NMR ($CDCl_3$) δ 8.82 (s, 1H), 8.3 (d, 1H), 7.38 (d, 1H), 6.94, 6.72 (s, 2H), 2.54 (s, 3H), 1.6−0.8 (m, 19H). Anal. ($C_{23}H_{28}O_3$) C,H.

Receptor Binding Assay. a. Binding to Synaptosomal Membranes. Synaptosomal brain preparations were made from whole rat brain as described previously.[26,27] Binding of [^{3}H]HU-243 (50.4 Ci/mmol) was assayed in triplicate as described.[26,27] In brief, each reaction mixture of 1 mL in siliconized Eppendorf tubes contained 2.4−3.8 μg of synaptosomal membrane protein, 28−48 fmol of [^{3}H]HU-243, and various concentrations of competing unlabeled cannabinoids. Tubes were incubated at 30 °C for 90 min and centrifuged at 13 000 rpm, and the tips of the tubes containing the pelleted membranes were cut and counted for their radioactivity.

b. Binding to Transfected COS-7 Cells. Two days after transfection (with 5 μg/100 mm of dish plasmids encoding CB_1 or CB_2) the cells were washed with phosphate-buffered saline, scraped, pelleted, and stored at −80 °C. Cell pellets were homogenized in binding buffer (50 mM Tris-HCl, 5 mM $MgCl_2$, and 2.5 mM EDTA, pH 7.4), and 50 μg protein aliquots were assayed for binding of [^{3}H]HU-243 as described above, except that the final concentration of [^{3}H]HU-243 was 300 pM. For more detailed information see refs 11 and 12.

Specific binding was defined as the difference between the amount of radioactivity bound to the pelleted membranes in the absence and presence of 50 nM unlabeled HU-243 (for a) or 1 μM unlabeled **5a** (HU-210) (for b) and was typically 70−80% of the total bound. The K_i values for the various cannabinoids and related compounds were calculated from the competition data according to the formula: $K_i = IC_{50}/1 + ([^3H]\text{-}HU\text{-}243/K_d)$.[29] The K_d values for HU-243 binding were 45[26] and 61 pM[11] for CB_1 and CB_2, respectively.

Cell Cultures. Cells were cultured in Dulbecco's modified Eagle's medium supplemented with 8% fetal calf serum, 2 mM glutamine, nonessential amino acids, 100 units/mL penicillin, and 100 μg/mL streptomycin in a humidified atmosphere consisting of 5% CO_2 and 95% air at 37 °C. CHO cells stably transfected with the cDNA of human CB_2 receptor[9] were described earlier.[11] COS-7 cells in 100 mm dishes were transiently transfected[12] with plasmids encoding rat CB_1[25] or human CB_2[9] (5 μg each) and, when indicated, with 2 μg of the plasmid containing the cDNA of adenylylcyclase type V.[12] Twenty-four hours later, the cells were trypsinized and cultured in 24-well plates. After an additional 24 h, the cells were assayed for adenylylcyclase activity. Transfection efficiency, determined by transfection with the cDNA for β-galactosidase, was 40−80%.

Adenylylcyclase Assay. The assay were performed as described.[11,12,27] Cells cultured in 24-well plates were incubated for 3 h with 0.25 mL/well fresh growth medium containing 5 μCi/mL [^{3}H]adenine. This medium was replaced with Dulbecco's modified Eagle's medium containing 20 mM Hepes (pH 7.4), 1 mg/mL fatty acid-free bovine serum albumin, 0.5 mM 1-methyl-3-isobutylxanthine, and 0.5 mM RO-20-1724. Cannabinoids and forskolin (1 μM) were added, and the cells were incubated at 37 °C for 10 min. The reaction was terminated with 1 mL of 2.5% perchloric acid containing 0.1 mM unlabeled cAMP. Aliquots of 0.9 mL were neutralized with 100 μL of 3.8 M KOH and 0.16 M K_2CO_3 and applied to a two-step column separation procedure.[12,27] The [^{3}H]cAMP was eluted into scintillation vials and counted. Background levels (cAMP accumulation in the absence of forskolin) were subtracted from all values and represented less than 10% of forskolin-stimulated cAMP accumulation.

Statistical Analysis. Data were anlyzed using the Student's *t*-test. Inhibition curves were generated with the Sigma Plot 4.11 program, and the EC_{50} values were determined using an equation from the ALLFIT program.[30]

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