Eur J Med Chem (1997) 32, 165–170

Short communication

Synthesis and pharmacology of (*R*)- and (*S*)-4'-hydroxy-Δ^9-tetrahydrocannabinols

M Singer[1], AK Dutta[1], DR Compton[2], BR Martin[2], RK Razdan[1]*

[1]*Organix, Inc, 65 Cummings Park, Woburn, MA 01801;*
[2]*Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298, USA*

(Received 29 May 1996; accepted 5 September 1996)

Summary — The (*R*)- and (*S*)-4'-hydroxy-Δ^9-tetrahydrocannabinols **1a,b** (tetrahydrocannabinol = THC) were synthesized by the condensation of (+)-*cis*-*p*-menth-2-ene-1,8-diol **3** with the corresponding (*R*)- and (*S*)-4'-acetoxy olivetols **4a,b**, followed by base hydrolysis. The optical purity of **1a,b** was estimated to be >87% ee. Pharmacological data presented here indicate that **1a** is two to three times more potent than **1b** in vivo (locomotion, antinociception and catalepsy measures) and eight times more potent in its affinity for the cannabinoid CB_1 receptor. Additionally, data from catalepsy and hypothermia measures at both the normal and short time-course evaluations indicate that both isomers possess a short duration of action which is presumably due to rapid metabolism. A comparison of the biological activity of 3'- and 4'-hydroxy-Δ^9-THCs indicates that although similar specificity resides in both the 3'- and 4'-positions, the 3'-position is more important than the 4'-position since (*S*)-3'-hydroxy-Δ^9-THC is much more potent than (*R*)-4'-hydroxy-Δ^9-THC. Furthermore **1b** has an indication of a lead for separation of hypothermic effects from other pharmacological activities.

cannabinoid / (*R*)- and (*S*)-4'-hydroxy-Δ^9-tetrahydrocannabinol / hypothermia / catalepsy

Introduction

Δ^9-Tetrahydrocannabinol (Δ^9-THC) is the main active constituent of marihuana and it is well established that various hydroxy metabolites of Δ^9-THC contribute to the full spectrum of pharmacological effects displayed by the parent compound [1–8]. We have previously reported on the synthesis and pharmacology of (*R*)- and (*S*)-3'-hydroxy-Δ^9-THCs **2b,a** and shown that the main cannabinoid activity resides in the *S*- rather than the *R*-isomer [9, 10]. This study was initiated because of our previous observations that the *R/S*-3'-hydroxy-Δ^9-THC was more potent than Δ^9-THC in various pharmacological tests including drug discrimination paradigm. It was shown that in all tests, except hypothermia, the *S*-isomer **2a** was clearly more potent than the *R*-isomer **2b**. The latter was approximately five to seven times less potent than the *S*-isomer in producing hypoactivity, the Δ^9-THC-like discriminative cue, and dog-ataxia. However in the case of hypothermia the two isomers appeared to be equiactive and were as potent as Δ^9-THC. The reason for this difference is not clear and it may be that hypothermia is mediated through a mechanism different from that responsible for the other central effects. Based on the observed stereospecificity of the 3'-hydroxy-THCs, we have speculated that the side-chain interacts with a lipophilic region of the cannabinoid receptor through hydrophobic bonding. Since side-chain hydroxylation is not essential for cannabinoid activity, it may be that the configuration of the side-chain in the *S*-isomer permits binding to the receptor whereas in the *R*-isomer it interferes with the interaction with the receptor [10]. In addition, these findings were considered to be important in terms of establishing the cannabinoid pharmacophore employing a comparative molecular field analysis (COMFA) of pharmacological potencies and binding affinities of cannabinoids. In this model, in addition to activity prediction based on the standard steric and electrostatic interactions, a hydrophobic field has been incorporated into COMFA for prediction of hydrophobic/hydrophilic interactions and their effect on activity. The model, incorporating 46 classical and non-classical cannabinoids has already been demonstrated to possess excellent predictive properties [11, 12].

*Correspondence and reprints

With this background, we considered it important to determine if similar specificity resides in the *R*- and *S*-isomers of 4'-hydroxy-Δ^9-THC **1**, a known metabolite of Δ^9-THC[1] (fig 1). It is noteworthy that in the Δ^8-series, the racemic 4'-hydroxy-Δ^8-THC was found to be nearly equiactive with the corresponding 3'-hydroxy-Δ^8-THC [4].

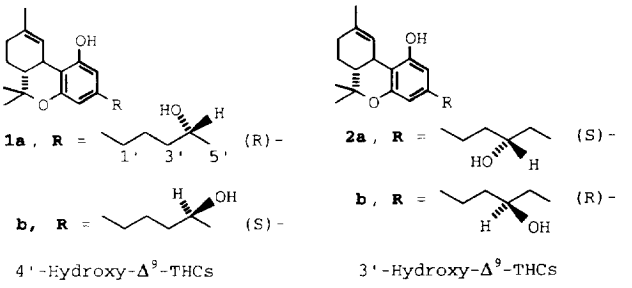
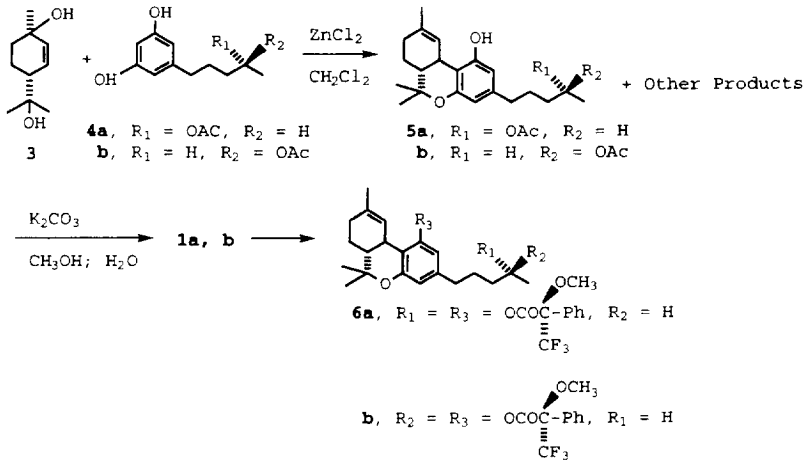


Fig 1. Structures of compounds **1a,b** and **2a,b**.

Chemistry

The (*R*)- and (*S*)-4'-hydroxy-Δ^9-THCs **1a,b** were synthesized (scheme 1) by the condensation of commercially available (+)-*cis*-*p*-menth-2-ene-1,8-diol **3** with the appropriate olivetol in the presence of $ZnCl_2$ using the procedure described earlier in the preparation of 3'-hydroxy-Δ^9-THCs **2** [9]. The synthesis of (*R*)- and (*S*)-4'-acetoxy olivetols **4a,b** from the known (*R*)- and (*S*)-1,2-epoxypropanes has already been reported [13]. The 4'-acetoxy-Δ^9-THCs **5a,b** thus obtained were hydrolyzed to give the 4'-hydroxy-Δ^9-THCs **1a,b**. The optical purity of **1a,b** was determined by NMR, using the Mosher ester **6a,b** [14]. They were prepared by using (*R*)-(+)-α-methoxy-α-trifluoromethylphenylacetic acid in the presence of dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) followed by purification by chromatography. A clear difference in chemical shifts between the two isomers was observed for the aromatic and the benzylic (C-1') protons. The chiral methine did not show clearly distinguishable signals between the two compounds (δ 5.16 and 5.15). The observed chemical shifts for the enantiomers are as follows: ArH, appeared as two singlets at δ 6.35 and 6.48 in the *R*-compound and at δ 6.39 and 6.54 in the *S*-compound; the $ArCH_2$ (t) signals were observed at δ 2.44 and 2.51 in the *R*- and *S*-compounds respectively.

It is noteworthy that the benzylic protons at C-1' are better separated in the Mosher ester **6a,b** (δ 2.44 and 2.51) compared to the *R*- and *S*-alcohols **1a,b** (δ 2.42 and 2.46). This is most probably due to the long-range influence of the chiral center in the Mosher esters. A similar effect has been reported by Keinan et al [15] in the Mosher esters of (*R*)- and (*S*)-8-hydroxynonan-2-ones. On the basis of NMR signals of the aromatic and the benzylic (C-1') protons we estimated that the optical purity of **1a** was 90% ee and **1b** was 88% ee. We are aware that for pharmacological work it would have been better to have a greater enantiomeric purity than what we achieved. However, we were unable to improve on it as the enantiomeric purity is dictated by the purity of the 1,2-epoxypropanes used in the initial stages of the multistep synthesis of **1a** and **1b**.



Scheme 1.

Table I. Dose–response data (mean ± SEM) for analogs **1a** and **1b** and time of evaluation.

Compound	*Dose (mg/kg)*	*Locomotion (counts) (5–15 min post-injection)*	*Antinociception (% MPE) (20 min post-injection)*	*Hypothermia (Δ °C) (60 min post-injection)*	*Catalepsy (% immobilization) (90–95 min post-injection)*
1a	0	1828 ± 44	11 ± 4	–0.1 ± 0.2	0 ± 0
	1	1547 ± 193	16 ± 9	–0.3 ± 0.4	1 ± 1
	3	1340 ± 479	3 ± 5	–1.3 ± 0.3*	1 ± 1
	10	542 ± 135*	82 ± 9*	–1.4 ± 0.2*	1 ± 1
	30	444 ± 152*	100 ± 0*	–1.9 ± 0.3*	24 ± 4*
	60	189 ± 20*	100 ± 0*	–3.9 ± 0.5*	47 ± 9*
1b	0	1298 ± 281	9 ± 4	–0.0 ± 0.4	0 ± 0
	3	1264 ± 281	8 ± 3	–0.2 ± 0.2	1 ± 1
	10	984 ± 327	52 ± 17*	–0.1 ± 0.9	7 ± 3
	30	327 ± 37*	85 ± 10*	–2.9 ± 1.1*	26 ± 12*
	60	411 ± 90*	78 ± 14*	–1.5 ± 0.3	12 ± 5

*Mean values (n = 6–12) significantly different ($P < 0.05$) versus control.

Pharmacology

The data generated for analogs **1a** and **1b** in the mouse model are provided in table I. A dose-responsive effect of **1a** was observed on all four measures of the mouse model of cannabimimetic activity. Sigmoidal curve fitting analysis indicated that the maximum effects as measured by locomotion, antinociception, hypothermia, and catalepsy were 90% inhibition, 100% MPE, –5.0 °C, and 60% immobility. These values have previously also been found to be representative of the maximum effect produced by Δ^9-THC and related cannabinoid analogs [16, 17] where more complete dose–response curves have been obtained. The ED_{50} values for **1a** on the tetrad of behaviors, respectively, were 4.7, 6.5, 36, and 36 mg/kg. Additionally, the dose–response curves were found to be parallel to one another, suggesting a similar molecular mechanism was involved. However, the difference in potency between the first two measures and the last two measures was rather unusual. For example, a dose of 10 mg/kg of **1a** produced pronounced antinociception and an inhibition of spontaneous locomotor activity, yet failed to produce any degree of catalepsy. This suggests either a separation of pharmacological effects, or indicates a limited duration of activity, observed in the last two measures (evaluated at 60 and 90 min post-injection) which may have been compromised by an unusually rapid metabolism of this cannabinoid analog.

The results obtained with **1b** indicate that this compound is less potent than **1a.** In the evaluation of locomotor activity a dose of 10 mg/kg of **1a** produced 70% inhibition, while **1b** produced a statistically non-significant 24% inhibition. Similarly, at 10 mg/kg **1b** produced less effect on the antinociception measure (52%) than did **1a** (82%). Additionally, **1b** failed to produce a dose-responsive effect in either the hypothermia or catalepsy measures. However, the great decrease in activity in the 60 and 90 min evaluations once again suggested that rapid metabolism might be a complicating factor in the normal evaluation of these two analogs. The ED_{50} values for **1b** on the locomotion and antinociception measures were 17 and 11 mg/kg.

The in vivo results suggest a potency difference between **1a** and **1b** of two to three times (locomotion and antinociception), which is substantiated by the in vitro affinity of these two analogs for the cannabinoid CB_1 receptor. The K_i values for **1a** and **1b** were 190 ± 36 and 1492 ± 177 nM, respectively. Thus, the in vitro data of **1a** and **1b** suggests that **1a** is approximately eight times more potent than **1b** in the binding affinity. Bearing in mind that **1a** and **1b** have optical purities of 90 and 88% ee respectively, it seems that the difference in affinity between **1a** and **1b** would be even greater in their optically pure forms. We do not have a pharmacological explanation for the low correlation noted between the binding affinity and the pharmacological effects. However, one cannot rule out the possibility of a contribution in part due to non-specific effects.

The endogenous cannabinoid anandamide has been shown to possess a very short duration of action in the

tetrad of measures of the mouse model of cannabinoid activity [17]. In that situation, the time of evaluation was altered such that tail-flick antinociception was evaluated at 5 min post-injection, immediately before locomotion was evaluated (at the typical time of 5–15 min post-injection). Animals were evaluated for hypothermia at 5 min post-injection and immediately evaluated for catalepsy in the ring-immobility procedure at 5–10 min post-injection. Thus, all evaluations were performed within 15 min of injection, as opposed to 90 min (as in the routine procedure for Δ^9-THC). Since the data in table I suggest that the evaluations of hypothermia and catalepsy might have been compromised by rapid metabolism, then these two measures of the tetrad were re-evaluated using the short time-course procedure developed for anandamide. These data are reported in table II.

The evaluation of **1a** and **1b** using the short-time course protocol indicated that each compound was more potent at 5–15 min than at 90 min. As indicated in table II, even the smallest dose evaluated (3 mg/kg) produced a statistically significant effect. The ED_{50} values for **1a** and **1b** in the catalepsy measure were 3.5 and 7.6 mg/kg. ALLFIT analysis also indicated the two dose–response curves were parallel, and the E_{max} was 78 ± 4% for both analogs, which is greater than that normally attained for Δ^9-THC. The potency of **1a** was 2.2 times that of analog **1b**, which corresponds well with that observed in the in vivo (two or three times) and in vitro (eight times) procedures.

Evaluation of hypothermia in table II indicated very similar effects for **1a** to that observed previously (table I), though possibly suggesting the maximal effect at 5 min was less than that normally observed for cannabinoids at 60 min. Thus, comparison of the ED_{50} values obtained at the two different times for **1a** should be done with caution. Nevertheless, it is clear that compound **1a** is pharmacologically active in the hypothermia measure, and generally more potent sooner after drug administration than later. These data also suggest that **1a** possesses a short duration of action, probably because it is rapidly metabolized.

The 5 min data confirm that **1b** is unable to produce a dose-responsive decrease in core temperature. Thus, the difference in potency between **1a** and **1b** in the hypothermia measure is not an indication of difference in metabolism. On the other hand, it may be indicative of some separation of pharmacological activity bearing in mind the enantiomeric purity of the compound. It is noteworthy that compound **1b** is the first cannabinoid evaluated which is somewhat ineffective in the production of hypothermia at doses capable of producing strong decreases in locomotor activity and in producing antinociception and catalepsy.

Table II. Dose–response data (mean ± SEM) for analogs **1a** and **1b** and time of evaluation.

Compound	*Dose (mg/kg)*	*Hypothermia (Δ °C) (5 min post-injection)*	*Catalepsy (% immobilization) (5–10 min post-injection)*
1a	0	–0.8 ± 0.2	1 ± 1
	3	–2.2 ± 0.2*	35 ± 5*
	10	–2.7 ± 0.2*	63 ± 4*
	30	–2.7 ± 0.3*	71 ± 3*
	60	–2.5 ± 0.2*	81 ± 4*
1b	0	–0.1 ± 0.2	1 ± 1
	3	–0.3 ± 0.3	16 ± 4*
	10	–1.2 ± 0.2*	50 ± 4*
	30	–0.7 ± 0.3	63 ± 3*
	60	–0.9 ± 0.3*	72 ± 5*

*Mean values (n = 6–12) significantly different ($P < 0.05$) versus control.

Conclusions

Though side-chain hydroxylation is not essential for cannabinoid activity, it appears that the configuration of the side-chain, which can be altered by hydroxylation, can interfere with the interaction of agonists with the receptor. Previous data suggested that in the 3'-hydroxy series, the *S*-isomer was more potent than the *R*-isomer. Data presented here indicate that a reversed specificity exists for the isomers of 4'-hydroxy-Δ^9-THC. (*R*)-4'-hydroxy-Δ^9-THC **1a** is two to three times more potent than the *S*-isomer in vivo (locomotion, antinociception, and catalepsy measures) and eight times more potent in its affinity for the cannabinoid CB_1 receptor. The reversed specificity of these compounds in their ability to produce cannabinoid-like pharmacological responses is consistent with previous data since, as shown in figure 1, the orientation of the (*R*)-4'-hydroxyl group **1a** and the (*S*)-3'-hydroxyl group **2a** is the same in space and they occupy a similar orientation in space in relation to the geminal methyls and the C-9 methyl of Δ^9-THC. Additionally, data from catalepsy and hypothermia measures at both the normal and short time-course evaluations indicate that both isomers possess a short duration of action, which is presumably due to rapid metabolism. However, when one compares the activity of the 3'-hydroxy-Δ^9-THCs to the 4'-hydroxy-Δ^9-THCs we conclude that although similar specificity resides in both the 3'- and 4'-positions the 3'-position is more important than the 4'-position since the (*S*)-3'-hydroxy-Δ^9-THC is much more potent than the (*R*)-4'-hydroxy-Δ^9-THC [9, 10]. Moreover, as stated earlier,

the difference in potency between **1a** and **1b** in the hypothermia measure is not an indication of difference in metabolism but rather an indication of some separation of pharmacological activity. Clearly, further evaluation of a series of cannabinoids possessing side-chain modifications such as in **1b** will be necessary to confirm the validity of this lead.

Studies relating to molecular modeling and incorporation of these compounds in the model for the cannabinoid pharmacophore are ongoing and will be reported elsewhere.

Experimental protocols

Chemistry

^{1}H-NMR spectra were recorded on either a Bruker 100 or a Varian XL400 spectrometer using tetramethylsilane as an internal reference. Thin layer chromatography (TLC) was carried out on Baker Si 250F plates. Visualization was accomplished with either iodine vapor, UV exposure or treatment with phosphomolybdic acid (PMA). Preparative TLC was carried out on Analtech uniplates Silica Gel GF 2000 microns. Flash chromatography was carried out on Baker Silica Gel 40 mM. Elemental analyses were performed by Atlantic Microlab, Atlanta, GA. Gas chromatography was carried out on a Perkin Elmer 5200 instrument using a capillary column. The terpene(+)-*cis*-*p*-menth-2-ene-1,8-diol was supplied by Firmenich and Company, New York.

(R)-4'-Acetoxy-Δ^9-tetrahydrocannabinol ***5a***

In a nitrogen atmosphere, a solution of 1.31 g (7.7 mmol) of (+)-*cis*-*p*-menth-2-ene-1,8-diol **3** and 1.834 g (7.7 mmol) of (*R*)-4'-acetoxy-olivetol **4a** [13] in a mixture of 5 mL of freshly distilled dry THF and 40 mL of dry CH_2Cl_2 was added to a well-stirred slurry of 10.0 g (73.3 mmol) of freshly fused, anhydrous $ZnCl_2$ and 40 mL of dry CH_2Cl_2 over 1 h. The color of the solution began to darken after 10 min and was a dark red after 2 h. The reaction was stopped after 4 h by decanting the red organic solution from the colorless granular solid, concentrating it in a rotary evaporator, and taking up the residue in ether. This solution was combined with the ethereal extract of the solution obtained by dissolving the granular solid in H_2O. The combined organic extracts were washed several times with H_2O and finally with saturated brine, dried, and concentrated in vacuo.

The residue (2.46 g) was purified via column chromatography using 120 g of silica gel and eluted with solutions of 20% EtOAc/hexane followed by 40% and then pure EtOAc. Multiple products were collected, analyzed by GC and systematically repurified by column chromatography. Using this procedure, as described before [9], yielded 0.53 g (18.5%) of **5a** as a gum. Similarly, (*S*)-4'-acetoxy-Δ^9-THC **5b** was synthesized from (*S*)-4'-acetoxyolivetol **4b**.

(R)- and (S)-4'-Hydroxy-Δ^9-THCs ***1a,b***

The appropriate 4'-acetoxy-Δ^9-THC (350 mg, 0.94 mmol) was dissolved in a solution of 4 mL of MeOH and 0.3 mL of H_2O. Solid K_2CO_3 (470 mg, 2.67 mmol) was added and the reaction was allowed to stir overnight under nitrogen when all the starting material was consumed (TLC). The reaction was concentrated in vacuo and the aqueous layer was extracted five times with ether. The combined ether extract was washed with H_2O and brine, dried (Na_2SO_4) and evaporated in vacuo to yield a foam, 278 mg (89%).

Compound **1a** showed a single spot by TLC (3:7 EtOAc/hexane, R_f = 0.17); ^{1}H-NMR ($CDCl_3$) δ, 6.33 (brs, 1H, H-10), 6.21, 6.11 (2s, 2H, aromatic), 3.78 (m, 1H, H-4'), 3.47 (m, 1H, H-10a), 2.42 (t, 2H, H-1'), 1.64 (s, 3H, H-11), 1.38 (s, 3H, H-6β), 1.15 (d, 3H, *J* = 6 Hz, H-5'), 1.08 (s, 3H, H-6α). Anal $C_{21}H_{30}O_3 \cdot 0.6H_2O$ (C, H). The presence of water was confirmed by NMR.

Compound **1b** showed a TLC exactly the same as **1a**. ^{1}H-NMR ($CDCl_3$) δ, 6.32 (brs, 1H, H-10), 6.24, 6.13 (2s, 2H, aromatic), 3.80 (m, 1H, H-4'), 3.48 (m, 1H, H-10a), 2.46 (t, 2H, H-1'), 1.67 (s, 3H, H-11), 1.41 (s, 3H, H-6β), 1.18 (d, 3H, *J* = 6 Hz, H-5'), 1.09 (s, 3H, H-6α). Anal $C_{21}H_{30}O_3 \cdot 0.4H_2O$ (C, H). The presence of water was confirmed by NMR.

Mosher diester ***6a,b***

Mosher diester **6a** was prepared by treatment of a mixture of 10 mg (0.03 mmol, 1 equiv) of **1a**, 39 mg (0.094 mmol, 3.1 equiv) of DCC and 22 mg (0.09 mmol, 3 equiv) of DMAP with a solution of 43 mg (0.09 mmol, 3 equiv) of (*R*)-(+)-α-methoxy-α-trifluoromethylphenylacetic acid (Aldrich) in 10 mL of dry THF. The solution was stirred overnight under nitrogen. The solvent was removed in vacuo and the residue was purified by preparative TLC using 30% EtOAc/hexane mixture. The upper spot was collected to yield 20 mg (90%) of **6a** as a gum; ^{1}H-NMR ($CDCl_3$) δ 7.3–7.7 (m, 5H, phenyl), 6.48, 6.35 (2s, 2H, aromatic), 5.46 (brs, 1H, H-10), 5.16 (m, 1H, H-4'), 3.76 (OCH_3), 2.44 (t, 2H, H-1'), 1.40 (s, 3H, H-11), 1.37 (s, 3H, H-6β), 1.32 (d, 3H, *J* = 6 Hz, H-5'), 1.08 (s, 3H, H-6α). Based on the integration [14] of the ArCH_2 signals and the Ar*H* signals in the NMR of **6a**, the optical purity of **1a** was calculated to be 90% ee.

Similarly the Mosher diester **6b** was prepared from 20 mg (0.06 mmol) of **1b** and yielded 41 mg (91%) of **6b** as a gum; ^{1}H-NMR ($CDCl_3$) δ 7.3–7.7 (m, 5H, phenyl), 6.54, 6.39 (2s, 2H, aromatic), 5.47 (brs, 1H, H-10), 5.15 (m, 1H, H-4'), 3.76 (OCH_3), 2.51 (t, 2H, H-1'), 1.39 (s, 3H, H-11), 1.37 (s, 3H, H-6β), 1.24 (d, 3H, *J* = 6 Hz, H-5'), 1.08 (s, 3H, H-6α). Using the same procedure as in the case of **1a**, the optical purity of **1b** was calculated to be 88% ee.

Pharmacology

Male ICR mice (Harlan Laboratories, Dublin, VA) weighing 18–25 g were used in all experiments. The mice were maintained on a 14/10 h light/dark cycle with free access to food and water. Δ^9-THC, obtained from the National Institute on Drug Abuse, was always evaluated as a positive control during the assessment of novel compounds. All compounds were dissolved in 1:1:18 (emulphor/ethanol/saline) for in vivo administration. Emulphor (EL-620, a polyoxyethylated vegetable oil, GAF Corporation, Linden, NJ) is currently available as Alkmulphor. All drug injections were administered iv (tail vein) at a volume of 0.1 mL/10 g of body weight. Mice were acclimated in the evaluation room overnight without interruption of food and water. Following drug administration each animal was tested for effects on the following tetrad of procedures: spontaneous (locomotor) activity at 5–15 min, tail-flick latency (antinociception) response at 20 min, core (rectal) temperature at 60 min, and ring-immobility (catalepsy) at 90–95 min, as described elsewhere [12]. In some experiments, where indicated, a shorter time course was used for the in vivo evaluation process. Following drug administration animals were tested as follows: either tail-flick latency (antinocicep-

tion) response at 5 min and spontaneous (locomotor) activity at 5–15 min, or core (rectal) temperature at 5 min and ring-immobility (catalepsy) at 5–10 min, as described elsewhere [17].

Spontaneous activity
Inhibition of locomotor activity was accomplished by placing mice into individual activity cages (6.5 x 11 inches), and recording interruptions of the photocell beams (16 beams per chamber) for a 10 min period using a Digiscan Animal Activity Monitor (Omnitech Electronics Inc, Columbus, OH). Activity in the chamber was expressed as the total number of beam interruptions.

Tail-flick latency
Antinociception was assessed using the tail-flick procedure. The heat lamp of the tail-flick apparatus was maintained at an intensity sufficient to produce control latencies of 2 to 3 s. Control values for each animal were determined prior to drug administration. Mice were then re-evaluated following drug administration and latency (s) to tail-flick response was recorded. A 10 s maximum was imposed to prevent tissue damage. The degree of antinociception was expressed as the % MPE (maximum possible effect) which was calculated as:

$$\% \text{ MPE} = \left[\frac{(\text{test latency} - \text{control latency})}{(10 \text{ s} - \text{test latency})} \right] \times 100$$

Core temperature
Hypothermia was assessed by first measuring baseline core temperatures prior to drug treatment with a telethermometer (Yellow Springs Instrument Co, Yellow Springs, OH) and a rectal thermistor probe inserted to 25 mm. Rectal temperatures were also measured following drug administration. The temperature difference (°C) between values was calculated for each animal.

Immobility
Catalepsy was determined by a ring-immobility procedure. At 90 min post-injection, mice were placed on a ring (5.5 cm in diameter) that was attached to a stand at a height of 16 cm. The amount of time (s) that the mouse spent motionless during a 5 min test session was recorded. The criterion for immobility was the absence of all voluntary movements (excluding respiration, but including whisker movement). The immobility index was calculated as:

$$\% \text{ immobility} = \left[\frac{\text{time immobile (s)}}{\text{length of session (s)}} \right] \times 100$$

Mice that fell or actively jumped from the ring were allowed five such escapes. Following the fifth escape, the test for that animal was terminated and immobility was calculated as a percentage of time that it remained on the ring before being discontinued. Data from mice failing to remain on the ring at least 2.5 min were not included.

Receptor binding
[^{3}H]CP-55,940 (K_D = 690 pM) binding to P_2 membranes was conducted as described elsewhere [12], except whole brain (rather than cortex only) was used. Displacement curves were generated by incubating drugs with 1 nM of [^{3}H]CP-55,940. The assays were performed in triplicate, and the results represent the combined data from three individual experiments.

Statistical analysis
Statistical analysis of all in vivo data was performed by ANOVA with Bonferroni/Dunn post-hoc for comparison to vehicle using the StatView statistical package (Brainpower, Inc, Agoura Hills, CA). Differences were considered significant at the $P < 0.05$ level. ED_{50} values were determined using ALLFIT, a program for the simultaneous curve fitting of a family of sigmoidal curves, as described elsewhere [17]. Similarly ALLFIT analysis was used to verify that the maximal effects for compounds on locomotion, temperature, antinociception, and catalepsy were, respectively, 90% inhibition, minus 5 °C, 100% MPE, and 60% immobility, as described elsewhere [16]. Thus, the ED_{50} values indicate response levels of 45% inhibition, minus 2.5 degrees, 50% MPE, and 30% immobility. The K_i values for radioligand displacement assays were determined using EBDA (Equilibrium Binding Data Analysis; Biosoft, Milltown, NJ).

Acknowledgment

This work was supported by Grants DA-05488 and DA-03672 from the National Institute on Drug Abuse.

References

1 Widman M, Nordquist M, Dollery CT, Briant RH (1975) *J Pharm Pharmacol* 27, 842–848
2 Agurell S, Binder M, Fonseka K et al (1976) In: *Marihuana, Chemistry, Biochemistry, and Cellular Effects* (Nahas G, ed), Springer, New York, 141–155
3 Harvey DJ, Martin BR, Paton WDM (1978) In: *Recent Development in Mass Spectrometry in Biochemistry and Medicine* (Frigerio A, ed), Plenum, New York, 161–184
4 Ohlsson A, Agurell S, Leander K et al (1979) *Acta Pharm Suec* 16, 21–33
5 Widman M, Halldin M, Martin BR (1979) In: *Marihuana Biological Effects* (Nahas GG, Paton WDM, eds), Pergamon, Oxford, UK, 101–103
6 Ohlsson A, Widman M, Carlsson S, Ryman T, Strid C (1980) *Acta Pharmacol Toxicol* 47, 308–317
7 Halldin MM, Widman MV, Bahr CV, Lindgren JE, Martin BR (1982) *Drug Metab Dispos* 10, 297–301
8 Agurell S, Halldin M, Lindgren JE et al (1986) *Pharmacol Rev* 38, 21–43
9 Sard H, Duffley RP, Robertson LR, Razdan RK (1984) *Synthesis* 506–509
10 Martin BR, Kallman MJ, Kaempf GF, Harris LS, Dewey WL, Razdan RK (1984) *Pharmacol Biochem Behav* 21, 61–65
11 Thomas BF, Compton DR, Martin BR, Semus SF (1991) *Mol Pharmacol* 40, 656–665
12 Martin BR, Compton DR, Thomas BF et al (1991) *Pharmacol Biochem Behav* 40, 471–478
13 Singer M, Siegel C, Gordon PM, Dutta AK, Razdan RK (1994) *Synthesis*, 486–488
14 Dale JA, Dull DL, Mosher HS (1969) *J Org Chem* 34, 2543–2549
15 Keinan E, Sinha SC, Sinha-Bagchi A (1992) *J Org Chem* 57, 3631–3636
16 Ryan W, Singer M, Razdan RK, Compton DR, Martin BR (1995) *Life Sci* 56, 2013–2020
17 Smith PB, Compton DR, Welch SP, Razdan RK, Mechoulam R, Martin BR (1994) *J Pharmac Exp Ther* 270, 219–227