Synthesis and Pharmacological Evaluation of Ether and Related Analogues of Δ^8-, Δ^9-, and $\Delta^{9,11}$-Tetrahydrocannabinol

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The primary goal of this research was to synthesize a series of ether analogues of the cannabinoid drug class and to evaluate their agonist and antagonist pharmacological properties in either the mouse or the rat. Agonist and antagonist activity was evaluated in mice using a multiple-evaluation procedure (locomotor activity, tail-flick latency, hypothermia, ring immobility) and activity in rats determined in a discriminative stimulus paradigm. Additionally, novel analogues were evaluated for their ability to bind to the THC receptor site labeled by ^{3}H-CP-55,940. None of the cannabinoid analogues were capable of attenuating the effects of Δ^9-THC (3 mg/kg) in either the rat (doses up to 10 mg/kg) or in the mouse (doses up to 30 mg/kg). It also appears that the compounds with minimal in vivo activity are not mixed agonist/antagonists. These data would suggest that the phenolic hydroxyl is important for receptor recognition (binding) and in vivo potency. Additionally, cannabinoid methyl ethers previously considered inactive have been found to produce limited activity. Lastly, data suggest that $\Delta^{9,11}$-THC is more potent than previous reports indicated, and does possess pharmacological activity.

Δ^9-Tetrahydrocannabinol (Δ^9-THC; (−)-6a,10a(*R*,*R*)-trans-Δ^9-THC; 3-pentyl-6a,7,8,10a-tetrahydro-6,6,9-trimethyl-6*H*-dibenzo[*b*,*d*]pyran-1-ol; see Table I for structure) produces a characteristic psychotropic response in humans and a variety of specific behavioral alterations in laboratory animals. The effects Δ^9-THC include disruption of conditioned operant responding (monkey), static ataxia (dog), a discriminative stimulus cue (rat), and a spectrum of pharmacological responses in the mouse that appear to be unique to this drug class. A multiple-evaluation procedure has been used in mice to successfully determine cannabimimetic properties of novel drugs, and thus can also be used to evaluate the ability of novel drugs to attenuate the pharmacological effects of Δ^9-THC.[1-3]

Although one mechanism of action of Δ^9-THC has been hypothesized to be a THC receptor, there currently is no strong evidence for the existence of a specific THC antagonist. The existence of such a compound is crucial in determining whether the ligand binding site described by Devane et al.[4] and Herkenham et al.[5] is in fact a receptor via which one or more cannabimimetic responses are produced. There has been one report[6] that the acid metabolite of Δ^9-THC will attenuate the cataleptic effects of the cannabinoids, which suggests a specific antagonist may exist. Though some reports have suggested that a variety of drugs (e.g. cannabidiol, cannabinol, phenitrone, imipramine, and amphetamine) attenuate the effects of Δ^9-THC, subsequent studies have failed to find antagonism by these compounds or to find that the attenuation of the THC-induced effect was simply the summation of opposite pharmacological responses rather than a direct effect.[7-14] One partial success in the quest for an antagonist is the fact that $\Delta^{9,11}$-THC was found to significantly reduce the effect of Δ^9-THC in the monkey.[1,15]

Since there are no general guidelines for designing an antagonist in a particular class of compounds, one possible approach to the development of a specific cannabinoid antagonist is to modify the structure of Δ^9-THC sufficiently to prevent activation (of the receptor site) without altering recognition. One of the simplest modifications described has been the conversion of the phenolic hydroxyl of Δ^9-THC to a methyl ether. Both methyl ethers of Δ^8- and Δ^9-THC have been found to be inactive in the monkey at doses up to 10 mg/kg, while Δ^9-THC produced prominent effects at 0.10–0.25 mg/kg.[16] Furthermore, the methyl ether of Δ^9-THC (4) was shown to be 25 times less potent than Δ^9-THC in the dog ataxia test.[37] However,

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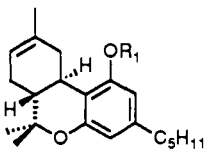
0022-2623/91/1834-3310$02.50/0

these ether compounds were not evaluated in rodents and have never been evaluated for potential antagonistic properties. Therefore, it was necessary to examine the ethers of Δ^8-, Δ^9-, and $\Delta^{9,11}$-THC for agonist activity in the rodents prior to the investigation of their antagonistic properties. (Structures for these three geometric isomers are presented in Table I.) Additionally, a few novel ethers were synthesized as preliminary probes to ascertain whether an ether linkage at the phenolic site could impart any antagonistic properties to the THC's. The biphenyl ethers (**5** and **6**) were synthesized since this substituent has previously been shown to impart antagonistic properties to compounds in the prostaglandin class of drugs,[38] and because evidence suggests some cannabinoid effects may be mediated by the prostaglandins.[17-19] The aminoalkyl ethers (**8–10**) were chosen to provide a nucleophilic site attached to the THC molecule (in place of a phenol) which could possibly interact with the receptor. The length of the chain was varied to facilitate this potential interaction. The morpholinoalkyl ether (**11**) was synthesized since the presence of an electron rich site, such as the oxygen of the morpholine, could possibly result in a mixed agonist/antagonist, as morpholino alkyl esters of THC's are known to be very potent agonists.[25]

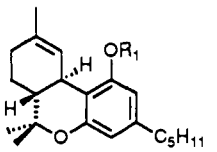
Since $\Delta^{9,11}$-THC was found to reduce the effect of Δ^9-THC[1,15] (see above), two modifications were performed in anticipation that this attenuation might be enhanced. First, the side chain was shortened to reduce the agonistic activity of $\Delta^{9,11}$-THC, resulting in compound **12**. Also, compound **1** was synthesized with a hydroxyl group in the $\Delta^{9,11}$-THC molecule. The rationale for this is based upon analogy to the opiate field, where it is well known that the addition of a hydroxyl group (at C-14) to morphine imparts antagonistic properties.

The primary goal of this research was to synthesize a series of ether analogues of the cannabinoid drug class and to evaluate their agonist and antagonist pharmacological properties. Additionally, agonist and antagonist pharmacological evaluation of the previously synthesized methyl ether analogues of Δ^8-, Δ^9- and $\Delta^{9,11}$-THC was performed. The models used to evaluate agonist and antagonist properties of cannabinoids included the mouse multiple-evaluation procedure and rat drug-discrimination paradigm. Additionally, in vitro displacement studies were performed to evaluate affinity to a THC receptor. This research constitutes an extension of previous work to purposely synthesize inactive or weakly active cannabinoids for the purpose of finding a specific THC antagonist.[20,21]

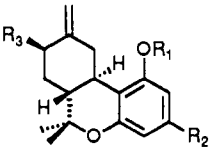
Table I. Tetrahydrocannabinol Derivatives



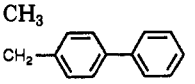
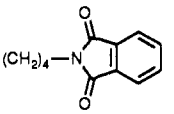
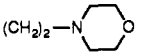
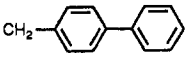
Δ^8-THC and derivatives



Δ^9-THC and derivatives



$\Delta^{9,11}$-THC and derivatives

compd	struct	R_1	R_2	R_3
Δ^8-THC	Δ^8	H		
3	Δ^8	CH_3		
5	Δ^8			
7	Δ^8			
8	Δ^8	$(CH_2)_4NH_2$		
9	Δ^8	$(CH_2)_3NH_2$		
10	Δ_8	$(CH_2)_8NH_2$		
11	Δ^8			
Δ^9-THC	Δ^9	H		
4	Δ^9	CH_3		
$\Delta^{9,11}$-THC	$\Delta^{9,11}$	H	n-C_5H_{11}	H
1	$\Delta^{9,11}$	H	n-C_5H_{11}	OH
2	$\Delta^{9,11}$	CH_3	n-C_5H_{11}	H
6	$\Delta^{9,11}$		n-C_5H_{11}	H
12	$\Delta^{9,11}$	H	n-C_3H_7	H
13	$\Delta^{9,11}$	CH_3	n-C_3H_7	H

Chemistry

All novel cannabinoids were prepared as the (−)-enantiomers and possessed the same stereochemical designations as Δ^9-THC (see nomenclature above). Analogues **1–4** were synthesized by previously published methods.[16,22,23] Analogues **5** and **6** were prepared from (−)-Δ^8- and (−)-$\Delta^{9,11}$-THC by treatment with 4-(chloromethyl)biphenyl, anhydrous potassium carbonate, and sodium iodide in refluxing acetone. Analogue **7** was prepared by treatment of (−)-Δ^8-THC with (4-bromobutyl)phthalimide, anhydrous potassium carbonate, and sodium iodide in refluxing acetone. Analogue **7** was deprotected with hydrazine hydrate in refluxing ethanol to produce analogue **8**. Analogues **9** and **10** were prepared similarly using (3-bromopropyl)phthalimide and (6-bromohexyl)phthalimide, respectively. Analogue **11** was prepared from (−)-Δ^8-THC by treatment with 2-chloroethylmorpholine, anhydrous potassium carbonate, and sodium iodide in refluxing acetone. 5-Propylresorcinol[34] was prepared by a sequence of reactions from 3,5-dimethoxybenzoic acid by reaction

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Table II. Pharmacological Activity of Cannabinoids

compd	mice[a]				rats:[a]	
	locomotor activity	tail-flick latency	hypothermia	ring immobility	discriminative stimulus	in vitro IC_{50}[b]
Δ^8-THC	1.9	1.5	15.5	5.2	1.1	179
	(1.3–2.9)	(0.6–4.1)	(6.1–39)	(3.6–7.7)	(0.3–2.6)	(23)
3	>100	33	>100	>100	not	3200
	(no value)	(3–330)	(no value)	(no value)	determined	(200)
11[c]	>100	>100	>100	118.4[d,f]	>10	4300
	(no value)	(no value)	(no value)	(no value)	(no value)	(600)
Δ^9-THC	1.0	1.4	1.4	1.5	0.6	218
	(0.5–1.4)	(0.4–3.2)	(0.9–4.4)	(0.4–3.6)	(0.3–1.7)	(37)
4	>100	116[d,f]	267[d,f]	17	>10	10000[e]
	(no value)	(no value)	(no value)	(5–55)	(no value)	(no value)
$\Delta^{9,11}$-THC	15	5.4	56	24	>30	334
	(11–22)	(4.2–6.9)	(41–76)	(9–65)	(no value)	(78)
1	20.0	25	50	63	>10	1200
	(11–47)	(8–71)	(15–150)	(18–223)	(no value)	(100)
12	20.0[f]	15[f]	>30	>30	>10	1400
	(no value)	(no value)	(no value)	(no value)	(no value)	(100)

[a] All in vivo values given as milligrams/kilogram with 95% confidence limits indicated parenthetically; ED_{50} values provided for all tests except MPE_{50} for tail-flick latency. [b] IC_{50} (nM) values determined against 1 nM ligand with SEM indicated parenthetically. [c] Partial activity only observed at one dose: 11 produced stimulation of locomotor activity (78%) and tail-flick (46% MPE) at 10 mg/kg. [d] Values estimated by extrapolation of probit analysis beyond highest dose evaluated (100 mg/kg). [e] Displacement not concentration dependent; concentration given produced 59% inhibition. [f] Confidence limits can not be determined on log dose–response regressions with two doses producing statistically significant effects.

with ethyllithium and reduction of the resulting ketone with hydrazine hydrate and potassium hydroxide in ethanol followed by demethylation of the methyl ethers (HI, acetic anhydride). Condensation of 5-propylresorcinol with *p*-menthenediol in benzene and PTSA (*p*-toluenesulfonic acid monohydrate) gave the (–)-Δ^8-THC analogue[39] which was converted to the corresponding (–)-$\Delta^{9,11}$-THC derivative **12** and its methyl ether **13**.

Pharmacology and Discussion of Results

The structures of all compounds are shown in Table I, and the data on pharmacologically active analogues are shown in Table II. The ED_{50} values for the parent cannabinoids (Δ^8-, Δ^9-, and $\Delta^{9,11}$-THC) were determined, and these data are similar to those previously reported.[1,3] Δ^8-THC varies in potency compared to Δ^9-THC, being between 0.09 and 0.9 times as active in the mouse and 0.5 times as active in the rat. This generally corresponds to the nearly identical binding affinities of Δ^8-THC and Δ^9-THC (179 and 218 nM, respectively). $\Delta^{9,11}$-THC varies in potency compared to Δ^9-THC, being between 0.025 and 0.26 times as active in the mouse. This only partly corresponds to the fact that $\Delta^{9,11}$-THC is only 0.65 times as effective as Δ^9-THC in the binding assay (334 and 218 nM, respectively). Previous reports suggested that $\Delta^{9,11}$-THC was 20-fold less potent than Δ^9-THC in the mouse activity cage,[24] which is similar to the 15-fold figure observed in these data. However, $\Delta^{9,11}$-THC did not produce generalization (>80% drug-lever selection) in the rat, so ED_{50} values cannot be compared directly. It is possible that $\Delta^{9,11}$-THC might produce generalization at doses greater than 30 mg/kg, since approximately 50% drug-lever selection was observed at 30 mg/kg and was accompanied by response rate suppression (sedation). However, it is equally possible that $\Delta^{9,11}$-THC might not completely generalize at any dose. Thus, $\Delta^{9,11}$-THC is at least 50-fold less potent than Δ^9-THC in the rat. Although this analogue has never been evaluated in humans,[25] it is likely that an extremely high dose would be required to produce psychoactivity. Similarly, it is estimated that an intravenous dose of 12.5 mg/kg of $\Delta^{9,11}$-THC would be required in the monkey to produce a response equivalent to 0.25 mg/kg of Δ^9-THC, yet the largest dose to be evaluated was 5 mg/kg.[26,27] Not surprisingly, this dose produced no effect. Thus, the conclusion that $\Delta^{9,11}$-THC is nonpsychoactive may simply be due to the fact that sufficiently large doses have not been evaluated. However, it is of interest to note that $\Delta^{9,11}$-THC is only 4-fold less potent than Δ^9-THC in the production of antinociception in the mouse. Therefore, it may be possible to develop active analogues of $\Delta^{9,11}$-THC which could prove useful clinically for pain relief at doses devoid of undesirable behavioral effects.

Cannabinoids for which pharmacological activity has previously been reported include **1**, **3**, and **4**. Analogue **1** is a major metabolite of $\Delta^{9,11}$-THC,[14] and it is weakly active in the monkey (estimated to be 100-fold weaker than Δ^9-THC).[27] This 8β-OH analogue was synthesized in an attempt to mimic (in the cannabinoid field) the production of an opioid antagonist when a hydroxy (at C-14) is substituted into the basic structure of morphine. Although this analogue was found to be weak compared to Δ^9-THC, as suggested by its weak (1.2 μM) actions at the receptor, its potency difference only varied from 18- to 42-fold (not 100-fold[27]). This compound also failed to produce generalization in the rat; however, the highest dose tested was 10 mg/kg. In contrast to **1**, both **3** and **4** were inactive in the monkey up to 10 mg/kg.[26] Data in the mouse and rat generally support the contention that the methyl ether analogues **3** and **4** are essentially inactive. This is sup-

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ported by the fact that **3** binds to the receptor only weakly (3.2 μM; 15 times less potent than Δ^9-THC), while **4** produces displacement (59%) only at a concentration of 10 μM. However, interesting pharmacological properties were observed in the mouse. The methyl ether of Δ^8-THC was inactive at 100 mg/kg except for production of antinociception (ED_{50} = 33.4 mg/kg). Although **3** is 24-fold weaker than Δ^9-THC in the tail-flick procedure, it is over 100-fold weaker in the production of other effects. It is possible that further exploration of the antinociceptive structure activity relationship (SAR) of this ether could lead to clinically useful compounds or molecular probes for evaluating potential mechanisms of action. Similarly **4**, the methyl ether analogue of Δ^9-THC, was 100-fold weaker than Δ^9-THC in most mouse evaluations. Interestingly, this analogue, unlike the ether of Δ^8-THC, did not show significant activity in the tail-flick procedure, but rather did produce ring immobility at a dose only 10-fold larger than that of Δ^9-THC. However, it is not clear how the ether modification is responsible for these unusual pharmacological responses, since the parent compounds produce both effects at the same dose. The weak receptor binding of these drugs may suggest that the observed pharmacological activities are not mediated by the same mechanism by which Δ^9-THC produces these actions.

A novel analogue for which pharmacological activity was observed was **12**, a $\Delta^{9,11}$-THC analogue with a shortened (propyl) side chain. Since increasing the length of the side chain is known to increase agonist potency in the cannabinoids class of drugs, the side chain of **12** was reduced in an effort to minimize agonist potency. This effort was at least partially successful in that receptor binding (1.4 μM) was weak compared to Δ^9-THC. Activity was observed in the locomotor and antinociception assays at doses 10–20-fold greater than that required of Δ^9-THC. However, it is not clear that this is a true separation of pharmacological effects (versus hypothermia and ring immobility) since the highest dose evaluated was 30 mg/kg. However, it is interesting that only a portion of the pharmacological spectrum is obtained with this shortened side chain. Evaluation of a series of bicyclic cannabinoids also showed a partial production of the spectrum of effects at certain side chain lengths. A minimum length was required to produce any effect (antinociception), as the side chain length increased the full spectrum of effects was produced, and upon further lengthening again only one action was produced (antinociception).[36]

The novel analogues **2**, **6**, **7**, **11**, and **13** were evaluated at doses up to 100 mg/kg and were found to be devoid of pharmacological activity. Similar results were obtained with **5**, though doses of up to only 70 mg/kg could be evaluated. Unlike the methyl ether analogues of Δ^8- and Δ^9-THC, **2** the methyl ether analogue of $\Delta^{9,11}$-THC was inactive in vivo and possessed weak receptor interaction (1.2 μM). The two analogues **5** and **6**, synthesized in an attempt to mimic the production of antagonists (in the prostaglandin class of drugs) by use of biphenyl substituents, proved to be completely ineffective at binding to the receptor (IC_{50} values >10 μM), as did the related analogue **7**. The aminoalkyl ether analogues **8–10** were synthesized in an effort to substitute a variable-length nucleophile site for the phenolic hydroxy. These analogues could only be evaluated up to 30 mg/kg, since lethality (>50%) occurs at this dose. These data support the previously established contention that introduction of free amines to the basic structure of THC increases toxicity.[21] Thus, none of these novel cannabinoids were found to be active in the rat. However, it should be noted that certain analogues (**2**, **7**, and **11**) were capable of *stimulating* locomotor activity at one or more of the lower doses evaluated, but the effect was not dose responsive and, therefore, was not considered a specific pharmacological effect.

Following completion of all in vivo pharmacological evaluations, each of the analogues were evaluated for their ability to displace ^{3}H-CP-55,940 from its binding site. Scatchard analysis of CP-55,940 binding from five independent experiments indicates a K_D of 742 ± 45 pM (mean ± SEM) and a B_{max} of 4.1 ± 0.6 pmol/mg of protein. Both Scatchard and displacement studies were conducted (see Experimental Section) in the appropriate temperature and protein concentration ranges. The affinity of CP-55,940 for the receptor binding site is sufficiently high to allow use of filtration methods for separation of bound and free radioligand. The total binding of ligand was sufficiently small (less than 10%) to allow use of the standard approximation of setting "free" ligand equal the concentration of the total added. Though cannabinoids bind to glass under many conditions, no corrections of "free" concentrations were necessary in this assay, since essentially no binding occurs to glass under these conditions (silanized glass tubes, buffer containing 5 mg/mL BSA). Linear regression analysis of log concentration versus displacement data indicates that Δ^9-THC (IC_{50} = 218 ± 37 nM) and Δ^8-THC (IC_{50} = 179 ± 23 nM) have moderate affinity for the receptor site. In contrast, none of the novel derivatives described in Table I and II bind potently to the site labeled by CP-55,940. Even at concentrations of 10 μM, a 50% displacement of ligand could not be obtained with compounds **5–9** or **13**. Analogue **4** produced a maximum displacement of 59% at a concentration of 10 μM, but failed to do so in a concentration-responsive manner. Weak affinity for the binding site is suggested by the IC_{50} values obtained with the remaining compounds: **1** (1.2 μM), **2** (1.2 μM), **3** (3.2 μM), **10** (2.5 μM), **11** (4.3 μM), and **12** (1.4 μM).

The primary goal of this research was to refine the SAR of ether analogues of the cannabinoid drug class and to determine if novel inactive or weakly active cannabinoids were capable of antagonizing the pharmacological effects of Δ^9-THC. There are pharmacokinetic unknowns which present interpretative problems when using in vivo measures to assess antagonist properties. A compound might not be absorbed or may be metabolized so rapidly that no drug is present during the time the agonist (Δ^9-THC) is introduced. In these studies the compound in question was given 10 min prior to administration of Δ^9-THC, and in the mouse model, the responses were measured at times between 5 and 90 min postinjection, which is a wide time frame in which to observe diminution of effects. However, the combined use of the in vivo approach with the in vitro binding assay greatly increases the chances of correctly identifying an antagonist.

All novel compounds (**1–13**) were evaluated for antagonist activity in the mouse model at doses of 10 or 30 mg/kg (**5**, **6**, **7**, **13**). None of the analogues were capable of attenuating the effects of Δ^9-THC (3 mg/kg) in the mouse. A subset of compounds (**1**, **4**, **5**, **6**, **11**, and $\Delta^{9,11}$-THC) were selected for evaluation of activity in the rat (based upon agonistic results in the mouse and chemical structure). None of the compounds produced generalization in the rat. Antagonist activity was evaluated in the rat at doses of 3 mg/kg (**5**) or 10 mg/kg. None of the analogues were capable of attenuating the effects of Δ^9-THC (3 mg/kg) in the rat. Thus, no further attempts were made to evaluate the remaining novel analogues for either agonistic or antagonistic properties in the rat. Thus, it may be concluded

that none of these cannabinoids are antagonists. Since all analogues possessed no or very weak affinity for the receptor labeled by CP-55,940 then it can also be concluded that those compounds with minimal activity are not mixed agonist/antagonists. The inactive cannabinoids apparently do not act as antagonists because they possess no affinity for the cannabinoid receptor(s).

Conclusions

Cannabinoid methyl ethers previously considered inactive have been found to produce limited activity in the mouse, though the effect observed with the methyl ether of Δ^8-THC was different from that observed with the methyl ether of Δ^9-THC. Additionally, though a large dose might be required, data presented here suggest that $\Delta^{9,11}$-THC possesses pharmacological activity, and is more potent than previous reports indicated. In general, a correlation exists between activity in the mouse multiple-evaluation procedure and production of activity in the rat, though no analogues (either weakly potent or inactive) antagonized the effects of Δ^9-THC in either the mouse or the rat. The inactivity of these novel cannabinoids may be due to a failure in the recognition process at the cannabinoid receptor(s), as indicated by the displacement binding studies. Additionally, weakly active analogues were not found to possess mixed agonist/antagonist properties.

Experimental Section

Chemistry. The infrared spectra were recorded on a Perkin-Elmer Model 1320 spectrophotometer. The NMR spectra were measured on a Varian T-60 spectrometer and are reported in parts per million with respect to tetramethylsilane as an internal standard. Elemental analysis was preformed by Atlantic Microlab, Inc. (Norcross, GA). Where analyses are indicated by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. High-resolution mass spectra were obtained from the Mass Spectrometry Facility, Cornell University (Ithaca, NY). Low-resolution mass spectra was preformed by Oneida Research Services, Inc. (Whitesboro, NY). The 25% silver nitrate impregnated silica gel was prepared by adding a solution of 6 g of silver nitrate in 10 mL of H_2O to 20 g of silica gel in 30 mL of H_2O. The mixture was stirred and then 150 mL of methanol was added. The solvent was concentrated in vacuo. Another 150 mL of methanol was added and the solvent again concentrated in vacuo. The remaining white solid was heated in an oven at 110 °C for 2 days. Silver nitrate impregnated TLC plates were prepared by soaking normal silica gel plates in a solution prepared from 5 g of $AgNO_3$, 10 mL of CH_3CN, and 100 mL of EtOH for 10 min and then drying at 110 °C for 0.5 h.

(−)-8β-Hydroxy-$\Delta^{9,11}$-tetrahydrocannabinol (1). Analogue 1 was synthesized by a previously published method.[22] The 8β-isomer was separated from the mixture by flash chromatography (30% ethyl acetate/hexanes).

(−)-1-*O*-Methyl-$\Delta^{9,11}$-tetrahydrocannabinol (2). $\Delta^{9,11}$-THC was prepared using a modified literature procedure.[23a] Δ^8-THC (1.4 g) was dissolved in 1 L of 5% *p*-xylene/2-propanol. The solution was placed in an Ace glass photolysis apparatus and degassed by bubbling nitrogen through the solution. The solution was photolyzed (medium-pressure Canrad-Hanovia, 250-W quartz mercury-vapor lamp) until capillary GC (5% methyl phenyl silicone; 25 M, 0.53 mm i.d. column) showed no change (4.5 h) in the ratio of $\Delta^{9,11}$- to Δ^8-THC (ca. 9.4:1). The solvent was then concentrated in vacuo and the crude (2.2 g) first purified on 150 g of silica gel with 10% ethyl acetate/hexanes. Capillary GC analysis showed this purified product (750 mg, 53%) to be 83% $\Delta^{9,11}$-THC, 7% Δ^8-THC, and 6% of an unidentified product. This mixture was purified a second time on 25 g of 25% silver nitrate impregnated silica gel with 20% ethyl acetate/hexanes to give 600 mg (43%) of $\Delta^{9,11}$-THC as a colorless gum identical to an authentic sample (TLC, ^{1}H NMR, GC). GC analysis showed this material to be >96% pure $\Delta^{9,11}$-THC. A mixture of the above $\Delta^{9,11}$-THC (381 mg, 1.21 mmol), K_2CO_3 (393 mg), MeI (1.5 mL), and acetone (5 mL) was refluxed under N_2 for 20 h. The mixture was poured onto H_2O (50 mL) and extracted with hexanes (3 × 50 mL). The combined hexanes extracts were washed with alcoholic KOH (25 mL) and H_2O (25 mL). After drying (Na_2SO_4) and concentration in vacuo, 300 mg of crude product was obtained. This was purified on 25 g of silica gel with 5% ethyl acetate/hexanes to yield 280 mg (73%) of **2** as a colorless gum:[23b] ^{1}H NMR ($CDCl_3$) δ 0.9–2.6 (m, 19 H), 1.0 and 1.35 (2 s, 6 H, CMe_2), 3.5 (m, 1 H, H-10α), 3.5 (s, 3 H, OCH_3), 4.7 (br s, 2 H C=CH_2), 6.15 and 6.25 (2 s, 2 H, ArH); TLC R_f = 0.57 (5% ethyl acetate/hexanes). Anal. ($C_{21}H_{32}O_2$) C, H.

(−)-1-*O*-Methyl-Δ^8-tetrahydrocannabinol (3) and (−)-1-*O*-Methyl-Δ^9-tetrahydrocannabinol (4). Analogues **3**[16] and **4**[16] were prepared by methylation using the method described above for $\Delta^{9,11}$-THC.

(−)-1-*O*-(Biphenylylmethyl)-Δ^8-tetrahydrocannabinol (5). A mixture of Δ^8-THC (681 mg, 2.17 mmol), K_2CO_3 (703 mg), 4-(chloromethyl)biphenyl (460 mg, 1.05 equiv), NaI (81 mg, 0.25 equiv), and acetone (15 mL) was refluxed under N_2 for 2 days. After 1 day an additional 100 mg of 4-(chloromethyl)biphenyl and 20 mg of NaI were added. The mixture was poured onto H_2O (50 mL) and extracted three times with hexanes (50 mL). The hexanes extracts were washed with alcoholic KOH (25 mL) and H_2O (25 mL). After drying (Na_2SO_4) and concentration in vacuo, this crude product was dissolved in 150 mL of acetone, the solution was degassed with N_2, and 5 mL of NaOH was added. This mixture was stirred for ca. 12 h to remove excess 4-(chloromethyl)biphenyl. H_2O (150 mL) was added and the product was extracted three times with 100 mL of hexanes. After drying (Na_2SO_4) and concentration in vacuo, this crude yellow oil was purified on 150 g of silica gel with 2.5% diethyl ether/petroleum ether to yield 310 mg (30%) of **5** as a colorless gum: ^{1}H NMR ($CDCl_3$) δ 0.8–2.8 (m, 16 H), 1.05 and 1.35 (2 s, 6 H, CMe_2), 1.6 (br s, 3 H, CH_3C=C), 3.3 (br d, 1 H, J = ca. 14 Hz, H-10α), 5.0 (s, 2 H, OCH_2), 5.35 (br s, 1 H, C=CH), 6.35 (s, 2 H, ArH), 7.45 (m, 9 H, Ph–Ph); TLC R_f = 0.42 (2.5% diethyl ether/petroleum ether). Anal. ($C_{34}H_{40}O_2$) C, H.

(−)-1-*O*-(Biphenylylmethyl)-$\Delta^{9,11}$-tetrahydrocannabinol (6). Analogue 6 was prepared from $\Delta^{9,11}$-THC in 75% yield using the method described above for **5**: ^{1}H NMR ($CDCl_3$) δ 0.8–2.8 (m, 18 H), 1.0 and 1.3 (2 s, 6 H, CMe_2), 3.75 (br d, 1 H, J = 12 Hz, H-10α), 4.6 (br s, 2 H, C=CH_2), 5.05 (s, 2 H, OCH_2), 6.2 (s, 2 H, ArH), 7.5 (m, 9 H, Ph–Ph); TLC R_f = 0.42 (2.5% diethyl ether/petroleum ether). Anal. ($C_{34}H_{40}O_2$) C, H.

(−)-1-*O*-(4-Phthalimidobutyl)-Δ^8-tetrahydrocannabinol (7). A mixture of Δ^8-THC (604 mg, 1.92 mmol), K_2CO_3 (630 mg), (4-bromobutyl)phthalimide (650 mg, 1.2 equiv), NaI (130 mg), and acetone (15 mL) was refluxed under N_2 for 5 days. After cooling to room temperature, the mixture was poured onto H_2O (200 mL) and diethyl ether (50 mL). The aqueous layer was extracted with diethyl ether (2 × 50 mL). The combined diethyl ether layers were dried (Na_2SO_4) and concentrated in vacuo to yield 1.13 g of a yellow oil. This crude product was purified on 120 g of silica gel with 10% ethyl acetate/hexanes to yield 750 mg (76%) of a colorless oil: ^{1}H NMR ($CDCl_3$) δ 0.8–2.8 (m, 20 H), 1.1 and 1.35 (2 s, 6 H, CMe_2), 1.85 (br s, 3 H, CH_3C=C), 3.1 (br d, 1 H, J = 14 Hz, H-10α), 3.9 (m, 4 H, OCH_2 and NCH_2), 5.4 (br s, 1 H, C=CH), 6.3 and 6.35 (2 s, 2 H, ArH), 7.8 (m, 4 H, Ph(H)C(O)); TLC R_f = 0.29 (10% ethyl acetate/hexanes). Anal. ($C_{33}H_{41}NO_4$) C, H, N.

(−)-1-*O*-(4-Aminobutyl)-Δ^8-tetrahydrocannabinol (8). To 7 (281 mg, 0.547 mmol) in 10 mL of absolute EtOH was added hydrazine hydrate (80 μL, 3.0 equiv). The mixture was then refluxed for 5 h. After the mixture cooled to room temperature, 2 mL of 1 M HCl was added. The solution was then neutralized (pH = 7) with dilute Na_2CO_3. The mixture was extracted with diethyl ether (3 × 25 mL). The diethyl ether extracts were dried (Na_2SO_4) and concentrated in vacuo to yield 320 mg of an oily white solid. The crude product was purified on 16 g of silica gel with 50% ethyl acetate/hexanes to yield 110 mg (52% yield) of a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.0–3.8 (m, 20 H), 0.9 (t, 3 H, J = 6 Hz, CH_2CH_3), 1.05 and 1.3 (2 s, 6 H, CMe_2), 1.7 (br s, 3 H, CH_3C=C), 3.7 (br t, 2 H, J = 6 Hz, OCH_2), 5.4 (br s, 1 H, C=CH), 6.25 and 6.3 (2 s, 2 H, ArH); IR ν_{max} (film) 1100, 1150, 1425, 1575, 2800–3200 (br) cm^{-1}; CI-MS m/e 386 (M + 1), 315, 72. Anal. ($C_{25}H_{39}NO_2 \cdot 0.5H_2O$) C, H, N.

(-)-1-*O*-(3-Aminopropyl)-Δ^8-tetrahydrocannabinol (9). Analogue 9 was prepared from Δ^8-THC (50% yield for two steps) in the same manner described above for 8 and 7 using (3-bromopropyl)phthalimide: ^{1}H NMR ($CDCl_3$) δ 0.8–3.6 (m, 23 H), 1.05 and 1.35 (2 s, 6 H, CMe_2), 1.65 (br s, 3 H, CH_3C=C), 4.0 (br t, 2 H, J = 6 Hz, OCH_2), 5.35 (br s, 1 H, C=CH), 6.1 (br s, 2 H, ArH). Anal. ($C_{24}H_{35}NO_2$) C, H, N.

(-)-1-*O*-(6-Aminohexyl)-Δ^8-tetrahydrocannabinol (10). Analogue 10 was also prepared from Δ^8-THC (41% yield for two steps) in the same manner described above for 8 and 7 using (6-bromohexyl)phthalimide: ^{1}H NMR ($CDCl_3$) δ 0.8–3.4 (m, 28 H), 1.05 and 1.35 (2 s, 6 H, CMe_2), 1.65 (br s, 3 H, CH_3C=C), 3.95 (br t, 2 H, J = 6 Hz, OCH_2), 5.4 (br s, 1 H, C=CH), 6.2 and 6.25 (2 s, 2 H, ArH). Anal. ($C_{27}H_{41}NO_2$), C, H, N.

(-)-1-*O*-(2-Morpholinoethyl)-Δ^8-tetrahydrocannabinol (11). A mixture of Δ^8-THC (653 mg, 2.078 mmol), K_2CO_3 (1.7 g), NaI (140 mg, 0.25 equiv), *N*-(2-chloroethyl)morpholine hydrochloride (464 mg, 1.2 equiv), and acetone (15 mL) was refluxed under N_2 for 3 days. After cooling to room temperature, the solution was poured onto diethyl ether (50 mL) and H_2O (50 mL). The aqueous layer was extracted with diethyl ether (3 × 25 mL). The combined diethyl ether layers were dried (Na_2SO_4) and concentrated in vacuo. The crude product was purified on 50 g of silica gel with 20% ethyl acetate/hexanes to yield 11 (720 mg, 81%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 0.8–3.0 (m, 22 H), 1.05 and 1.3 (2 s, 6 H, CMe_2), 1.7 (br s, 3 H, CH_3C=C), 3.25 (br t, 1 H, J = ca. 14 Hz, H-10α), 3.7 (br t, 4 H, J = 5 Hz, CH_2OCH_2), 4.05 (br t, 2 H, J = 5 Hz, $ArOCH_2$), 5.4 (br s, 1 H, C=CH), 6.2 and 6.25 (2 s, 2 H, ArH); CI-MS m/e 342 (M + 1), 114, 100. Anal. ($C_{27}H_{41}NO_3$) H, N; C: calcd, 75.84; found, 76.50.

(-)-3-Norpentyl-3-propyl-$\Delta^{9,11}$-tetrahydrocannabinol (12). 5-Propylresorcinol was synthesized by a modification of a literature procedure.[34] To 3,5-dimethoxybenzoic acid (14.76 g, 81 mmol) in 200 mL of diethyl ether under N_2 at -78 °C was added 275 mL of 0.81 M ethyllithium (223 mmol, prepared from ethyl bromide and lithium) dropwise over 1 h. After stirring at -78 °C for 0.5 h, the reaction was warmed to 0 °C and stirred for 1 h and then stirred 18 h at room temperature. The reaction was carefully poured onto 1 L of 1 M HCl. The resulting aqueous layer was purified and extracted once more with diethyl ether (500 mL). The combined diethyl ether layers were washed with saturated $NaHCO_3$, dried (Na_2SO_4), and concentrated in vacuo. The crude solid was recrystallized from 250 mL of petroleum ether at -20 °C to obtain 3,5-dimethoxyphenyl ethyl ketone as a white solid (13.4 g, 85%, mp 33–36 °C, lit.[34] mp 33.5–34 °C): ^{1}H NMR ($CDCl_3$) δ 1.2 (t, 3 H, J = 7 Hz, CH_3), 2.85 (q, 2 H, J = 7 Hz, CH_2), 3.8 (s, 6 H, OCH_3), 6.6 (t, 1 H, J = 1 Hz, *p*-ArH), 7.1 (d, 2 H, J = 1 Hz, *o*-ArH).

A mixture of 3,5-dimethoxyphenyl ethyl ketone (5.3 g, 27.3 mmol), hydrazine hydrate (2.75 g, 56 mmol) and 10 mL of absolute ethanol was refluxed for 6 h under N_2. The ethanol and hydrazine hydrate were removed by distillation. KOH (11.2 g) was added and the mixture heated at 230 °C for 0.5 h. The mixture was distilled at 2 mmHg, and 3.4 g (69%) of 1,3-dimethoxy-5-propylbenzene was obtained (bp 92–94 °C, 2 mmHg, lit.[34] bp 103–105 °C, 3 mmHg): ^{1}H NMR ($CDCl_3$) δ 1.9 (t, 3 H, J = 7 Hz, CH_3), 1.6 (m, 2 H, CH_2CH_3), 2.5 (t, 2 H, J = 8 Hz, CH_2CH_2), 3.7 (s, 6 H, OCH_3), 6.3 (s, 3 H, ArH).

To 1,3-dimethoxy-5-propylbenzene (5.23 g, 29.0 mmol) in HI (70 mL) at 0 °C under N_2 was added Ac_2O (45 mL) dropwise. The solution was then refluxed for 1 h. After the solution cooled to room temperature, 58 g of $K_2S_2O_5$ in 200 mL of H_2O was added. The solution was extracted with diethyl ether (6 × 100 mL). The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The crude oil was purified on 375 g of silica gel with 40% ethyl acetate/hexanes to yield 3.97 g (90% yield) of an oil which crystallized at -20 °C to give a white solid, 5-propylresorcinol (mp 77–79 °C, lit.[34] mp 86–87 °C): ^{1}H NMR ($CDCl_3$) δ 1.8 (t, 3 H, J = 7 Hz, CH_3), 1.5 (m, 2 H, CH_2CH_3), 2.35 (t, 2 H, J = 7 Hz, CH_2CH_2), 6.15 (s, 3 H, ArH), 6.85 (s, 2 H, OH, D_2O exchangeable).

A mixture of 5-propylresorcinol (2.99 g, 16.59 mmol), *p*-menthene-1,8-diol[39] (3.0 g, 17.65 mmol), PTSA (533 mg), and benzene (100 mL) was refluxed with a Dean–Stark apparatus under N_2 for 2 h. The optical status of these reactants necessary to produce the desired (-) enantiomer has been defined previously.[39] After cooling to room temperature, the solution was poured onto saturated $NaHCO_3$ (200 mL). The aqueous layer was extracted once more with benzene. The combined benzene layers were dried (Na_2SO_4) and concentrated in vacuo. The crude product was purified on 500 g of silica gel with 10% ethyl acetate/hexanes to yield 3.05 g of 3-norpentyl-3-propyl-Δ^8-tetrahydrocannabinol: ^{1}H NMR ($CDCl_3$) δ 0.85 (t, 3 H, J = 7 Hz, CH_3CH_2), 1.05 and 1.35 (2 s, 6 H, CMe_2), 1.0–3.0 (m, 7 H), 2.35 (br t, 2 H, J = 7 Hz, CH_2CH_3), 3.1 (br d, 1 H, J = ca. 14 Hz, H-10α), 5.4 (s, 1 H, C=CH), 5.9 (br s, 1 H, ArOH), 6.05 and 6.3 (2 br s, 2 H, ArH).

3-Norpentyl-3-propyl-Δ^8-tetrahydrocannabinol was converted to 12 (26%) by the same procedure described for $\Delta^{9,11}$-THC: ^{1}H NMR ($CDCl_3$) δ 0.85 (t, 3 H, J = 6 Hz, CH_3CH_2), 1.05 and 1.35 (2 s, 6 H, CMe_2), 0.8–2.8 (m, 11 H), 3.75 (br d, 1 H, J = 12 Hz, H-10α), 4.75 (br s, 2 H, C=CH_2), 5.5 (s, 1 H, ArOH), 6.05 and 6.25 (2 d, 2 H, J = 1 Hz, ArH); CI-MS m/e 287 (M + 1); EI-MS m/e 286 (M^+), 271, 243, 203. Anal. ($C_{19}H_{26}O_2 \cdot 0.25H_2O$) C, H.

(-)-1-*O*-Methyl-3-norpentyl-3-propyl-$\Delta^{9,11}$-tetrahydrocannabinol (13). This analogue was prepared in 90% yield from 12 by the method described for 2: ^{1}H NMR ($CDCl_3$) δ 0.9 (t, 3 H, J = 6 Hz, CH_3CH_2), 1.05 and 1.3 (2 s, 6 H, CMe_2), 1.0–2.8 (m, 11 H), 3.5 (m, 1 H, H-10α), 3.7 (s, 3 H, OCH_3), 4.65 (br s, 2 H, C=CH_2), 6.1 and 6.15 (2 s, 2 H, ArH); TLC R_f = 0.6 (5% ethyl

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acetate/hexanes). Anal. ($C_{20}H_{28}O_2$) C, H.

Pharmacology. Materials. Male ICR mice (22–30 g) and Sprague-Dawley rats (250–275 g) obtained from Dominion Laboratories (Dublin, VA) were maintained on a 14:10-h light:dark cycle and received food and water ad libitum. Δ^8-, Δ^9-, and $\Delta^{9,11}$-THC were obtained from the National Institute on Drug Abuse as the (-) enantiomers. ^{3}H-CP-55,940 was kindly provided by Dr. Kenner C. Rice (Lab. Med. Chem./NIDDK, NIH, Bethesda, MD).

Drug Preparation and Administration. The procedure of Olson et al.[28] was used to prepare micellular suspensions suitable for injection, resulting in a final vehicle composition of ethanol:emulphor:saline (1:1:18), which was administered via tail-vein injection (0.1 mL/10 g, iv) to mice or intraperitoneally (0.1 mL/100 g, ip) to rats.

Behavioral Evaluations. Locomotor activity (% inhibition), antinociception (via tail-flick latency; expressed as %MPE), hypothermia (Δ °C), and catalepsy (i.e. ring immobility; expressed as % immobility) were evaluated in mice by previously reported methods.[3,20,21,29]

To establish the drug discrimination model in rats, animals were trained to discriminate between vehicle and Δ^9-THC (3 mg/kg, ip) 30 min postinjection. The protocol design was a slight modification[30,31] of the standard two-level operant procedure for a FR-10 schedule of food reinforcement.[32,33]

Antagonist properties of the cannabinoids were determined as described previously.[3,20,21,29] Animals were pretreated with drug 10 min prior to administration of 3 mg/kg Δ^9-THC, and all pharmacological evaluations were performed as described above.

Statistical analysis was performed using ANOVA with Dunnett's t test for comparisons to control (agonist evaluations), and the Scheffe's F test for multiple comparisons (antagonist evaluations). Differences were considered significant at the $p < 0.05$ level (two-tailed). The ED_{50} value for agonist activity was determined by unweighted least-squares linear regression of the log dose-probit analysis.

In Vitro Binding Assays. The filtration procedure used for ^{3}H-CP-55,940 binding is a modification of the centrifugation method described by others.[4] Five rats were decapitated and their cortices rapidly dissected free and homogenized in 30 mL of 0.32 M sucrose which contained 2 mM EDTA and 5 mM $MgCl_2$. The homogenate was centrifuged at 1600g for 10 min, and the supernatant was removed. The pellet was washed twice by resuspending in 0.32 M sucrose/2 mM EDTA/5 mM $MgCl_2$ and centrifuging again as described above. The original supernatant was combined with the wash supernatants and centrifuged at 39000g for 15 min. The resulting P_2 pellet was suspended in 50 mL of buffer (50 mM Tris·HCl, pH 7.0, 2 mM EDTA, 5 mM $MgCl_2$) and incubated at 37 °C for 10 min before centrifugation at 23000g for 10 min. The P_2 pellet was resuspended in 50 mL of 50 mM Tris·HCl/2 mM EDTA/5 mM $MgCl_2$ and incubated at 30 °C for 10 min before centrifugation at 11000g for 15 min. The final pellet was resuspended in 10 mL of 50 mM Tris·HCl (pH 7.4) which contained 1 mM EDTA and 3 mM $MgCl_2$ and then stored at -40 °C.

The binding assay was performed in silanized glass tubes which contained 100 μL of radiolabeled ligand (final concentration 1 nM), 100 μL of competing unlabeled drug, 150 μg of membrane protein (75 μL), and sufficient buffer (50 mM Tris·HCl, pH 7.4, 1 mM EDTA, 3 mM $MgCl_2$ and 5 mg/mL bovine serum albumin [BSA]), to make a final volume of 1 mL. After a 1-h incubation at 30 °C, the reaction was terminated by the addition of 2 mL of ice-cold 50 mM Tris·HCl (pH 7.4) buffer containing 1 mg of BSA/mL and rapid filtration through polyethylenimine-treated Whatman GF/C glass-fiber filters. The reaction tube was washed with a 2-mL aliquot of buffer, which was then also filtered. The filters were washed with two 4-mL aliquots of ice-cold buffer. The filters were shaken for 60 min in 10 mL of scintillation fluid, and radioactivity was quantitated by liquid scintillation spectrometry. Specific binding was defined as the difference between the binding that occurred in the presence and absence of 10 μM unlabeled CP-55,940.

Acknowledgment. This work was supported by NIDA Grants DA 03672 and DA 05488 and the Commonwealth of Virginia Center on Drug Abuse.

Synthesis and Biological Activity of the Putative Metabolites of the Atypical Antipsychotic Agent Tiospirone

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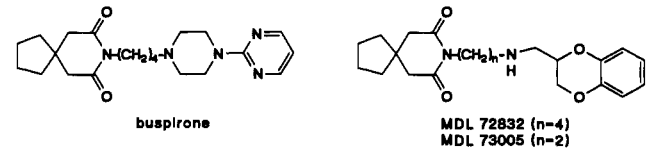
Putative oxidative metabolites of the lead antipsychotic agent tiospirone (1) were synthesized to assist in the identification of the authentic metabolic products found in human urine samples. Thus far, six authentic metabolites have been correlated to the synthetic species.[4a] The putative metabolites were further examined in vitro to assess their central nervous system therapeutic potential. SAR analysis of these derivatives indicates that hydroxyl substitution, particularly in the azaspirodecanedione region of the molecule, diminishes the dopamine D-2 affinity of the species without significantly altering the serotonin type-1A and type-2 interactions. In addition, an increase in α_1-adrenergic affinity appears to be linked to the attenuation of effects at the dopamine receptors. The biological profile of the 6-hydroxytiospirone metabolite **42** was exemplary in these respects and the in vivo actions of this compound suggest potent antipsychotic potential with a minimal liability for extrapyramidal side effects (EPS). While compound **42** has been unambiguously characterized as an actual human metabolite of tiospirone, the role of **42** in the observed antipsychotic activity of the parent drug, if any, has not yet been determined.

Introduction

On the basis of extensive preclinical studies and preliminary clinical evaluations, tiospirone (1, 8-[4-[4-(1,2-benzisothiazol-3-yl)-1-piperazinyl]butyl]-8-azaspiro[4.5]-decane-7,9-dione, a.k.a. tiaspirone or BMY 13859) is a lead compound from the azaspirodecanedione class of pharmaceuticals indicated for the treatment of psychotic dis-

Chart I



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0022-2623/91/1834-3316$02.50/0