to each cell. After 10 min, the trypsin was aspirated and placed in a centrifuge tube (15 mL) containing 1 mL of complete media (10% NBCS). This process was repeated for the content of each cell. The wells were washed with saline, and the washes were added to the tube. The tube was vortexed and then placed in the centrifuge and spun for 800*g* for 5 min. The media was decanted, and 1 mL of a 0.1% Trypsin blue in PBS was added to each tube. The tubes were vortexed, and the cells were counted with a hemocytometer.

Registry No. **2**, 88703-35-9; **3a**, 74254-89-0; **3b**, 88703-36-0; **3c**, 88703-37-1; **3d**, 88703-38-2; **3e**, 88703-39-3; **4**, 88703-40-6; decanoyl chloride, 112-13-0; dihydropyran, 110-87-2; octanoyl chloride, 111-64-8; propanoic anhydride, 123-62-6.

Synthesis and Pharmacological Activity of Some 9-Substituted Δ^8-Tetrahydrocannabinol Analogues[1]

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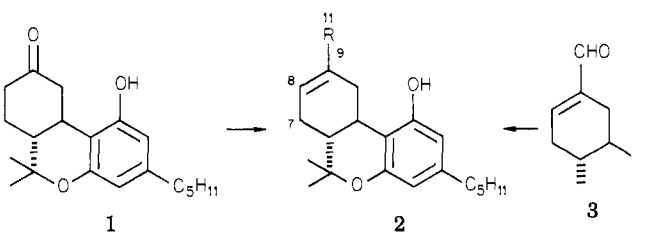
Several 9-substituted Δ^8-tetrahydrocannabinol (Δ^8-THC) analogues were synthesized and evaluated for biological activity in mice. Compounds with phenyl (**2b**) and butyl (**2c**) substituents were prepared by the addition of phenyllithium and *n*-butyllithium, respectively, to (−)-9-nor-9-oxohexahydrocannabinol (**1**), followed by dehydration, whereas, isopropyl (**2d**), $PhCH_2$ (**2e**), and $Ph(CH_2)_2$ (**2f**) derivatives were synthesized via the Grignard reaction with subsequent dehydration. Compounds with $C_2H_5CH(OH)$ (**2g**) and $CH_3CH(OH)$ (**2h**) substituents at C-9 were prepared from (−)-9-nor-9-formyl-Δ^8-tetrahydrocannabinol acetate (**3**) by the reaction of ethyl and methyl Grignard reagents, respectively. Biological activity indicated that a methyl group at the C-9 position is, thus far, optimum for producing hypoactivity and hypothermia in mice. In addition, hydroxyethyl substitution at position 9 reduced the antinociceptive activity of Δ^8-THC, in contrast to the increased activity reported for hydroxymethyl substitution.

The current interest in cannabinoids as potential therapeutic agents has given new direction to their structure–activity relationships (SAR). With the possibility that cannabinoids may eventually be useful in the treatment of a variety of diseases,[2] there is a need to develop cannabinoids that are more specific in their effects. It is well documented that Δ^9- and Δ^8-THC's are rapidly metabolized in vivo to their corresponding 11-hydroxy derivatives.[3] They are biologically potent with a pharmacological profile similar to the parent compounds. In addition, it has been reported that 9-nor-Δ^8-THC has a pharmacological profile similar to that of Δ^8-THC.[4]

In an attempt to further characterize the hydrocarbon substitution pattern at C-9 for optimum biological activity, we synthesized several 9-substituted Δ^8-THC analogues. They were evaluated for their ability to alter spontaneous activity, antinociceptive activity, and body temperature in mice in an effort to separate behavioral effects from other pharmacological effects. The results of these studies are presented in this paper.

Chemistry. The various Δ^8-THC analogues **2b** to **2h** were conveniently synthesized from either the known (−)-9-nor-9-oxohexahydrocannabinol (**1**)[5] or the (−)-9-nor-9-formyl-Δ^8-tetrahydrocannabinol acetate (**3**)[6] (Scheme I). The compounds **2b** and **2c** were prepared by the addition of phenyllithium and *n*-butyllithium, respectively, to the ketone **1**, followed by dehydration, whereas **2d** to **2f** were synthesized via the Grignard reaction (prepared from the appropriate halide), followed by dehydration. Compounds **2g** and **2h** were synthesized from **3** by the reaction of ethyl and methyl Grignard reagents, respectively, followed by careful decomposition with NH_4Cl to avoid dehydration.

Scheme I



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Pharmacological Results and Discussion

The discovery that the 11-hydroxy metabolite of Δ^9-THC exhibited potent cannabinoid activity[3a] served to demonstrate the importance of the methyl substituent at C-9. Similar results were observed in the Δ^8 series.[3b] Removal of the methyl group at C-9 in both the Δ^9 and Δ^8 series does not abolish behavioral activity but merely reduces it by 50–60%.[4] Similarly, replacement of the methyl by CH_2CH_3, CH_2OCH_3, or CH_2NH_2 at C-9 reduces but does not eliminate the behavioral activity in the Δ^8 series.[7] On the other hand, when the double bond is exocyclic ($\Delta^{9(11)}$) rather than endocyclic, as in Δ^9- or Δ^8-

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Table I. Effects of Δ^8-THC and Its Analogues on Spontaneous Activity and Rectal Temperature in Mice

compd	R[c]	spontaneous act.[a]	hypothermia[b] dose	hypothermia[b] Δ °C
vehicle				0.2 ± 0.2
Δ^8-THC (2a)	CH_3	7.1 (4.5-11.2)	5	0.9 ± 0.3
			10	1.0 ± 0.3
			20	3.4 ± 0.3
2b	Ph	67 (44-102)	30	1.6 ± 0.5
			60	0.3 ± 0.9
			100	2.2 ± 0.3
2c	*n*-butyl	32 (17-61)	10	0.3 ± 0.4
			30	4.3 ± 0.8
			60	5.0 ± 0.5
2d	*i*-propyl	26 (11-60)	20	1.2 ± 0.4
			30	0.8 ± 0.3
			60	1.3 ± 0.3
2e	$PhCH_2$	32 (10-101)	10	1.3 ± 0.2
			20	0.9 ± 0.2
			30	1.1 ± 0.2
			60	1.5 ± 0.3
2f	$Ph(CH_2)_2$	43 (27-68)	30	0.3 ± 0.6
			40	0.3 ± 0.3
			60	0.9 ± 0.3
2g	$C_2H_5CH(OH)$	39 (24-61)	10	0.4 ± 0.2
			30	−0.2 ± 0.2
			60	1.3 ± 0.4
2h	$CH_3CH(OH)$	27 (21-36)	20	0.0
			30	0.8 ± 0.2
			40	1.3 ± 0.4

[a] ED_{50}, mg/kg iv; 95% confidence limits are shown in parentheses. [b] Dose in milligrams per kilogram iv; Δ °C represents the difference between preinjection and postinjection (60 min) temperatures. [c] See Scheme I.

THC's, the behavioral activity is reduced dramatically.[3b,8] In order to further assess the pharmacological importance of substitutions at the C-9 position, the Δ^8-THC analogues **2b–h** were tested for their effects on spontaneous activity and body temperature in mice, and the results are given in Table I. All of the Δ^8-THC analogues were found to be 4–5 times less effective than Δ^8-THC in producing hypoactivity. The only exception was the phenyl derivative **2b**, which was almost 10 times less potent. The hypothermic effect was also less with all of these compounds, with the exception of the butyl derivative **2c**. The results in this case are probably anomolous, rather than representing a unique structural requirement for specific cannabinoid actions. From these data, it appears that a methyl group at the C-9 position is optimum for producing hypoactivity and hypothermia in the Δ^8 series.

The hydroxyethyl derivative (**2h**) was compared to Δ^8-THC for antinociceptive activity. The ED_{50} (CL) for Δ^8-THC in the tail-flick assay was 3.6 (1.2–11.2) mg/kg, 5 min after intravenous (iv) administration, whereas **2h** only produced 40% or less of the maximum possible effect at doses up to 20 mg/kg at 5, 10, and 15 min after treatment. Δ^8-THC was found to be almost 10 times more active than **2h** in the phenylquinone-stretching test, as evidenced by their respective ED_{50}'s (CL) of 0.56 (0.34–0.91) and 4.1 (2.9–5.7) mg/kg. Compound **2g** was not tested due to a limited supply. The 11-hydroxy metabolites of Δ^8-THC has been reported to be almost 5 times more active than Δ^8-THC in the mouse hot-plate test.[9] Although only one hydroxylated compound could be tested, the data suggest that increasing the size of the hydroxylated 9-substituent decreases antinociceptive activity and that, thus far, optimal activity resides in the 11-hydroxy metabolite.

Experimental Section

The NMR spectra were measured on a Varian T-60 spectrometer. The analyses by gas chromatography (GC) were performed on a Varian 6000 instrument equipped with a 6 ft × 0.125 in. i.d. stainless-steel column packed with 3% OV-17 on 80–100 mesh Supelcoport and a flame-ionization detector (240–260 °C). Preparative GC was carried out on a Varian Model 90P instrument equipped with 5 ft × 0.25 in. i.d. stainless-steel column packed with SE 30 on 100/120 Diatomite CLQ and a flame-ionization detector (245 °C). Where indicated, compounds were converted to their trimethylsilyl ethers (TMSE) with *N,N*-bis(trimethylsilyl)-2,2,2-trifluoroacetamide (BSTFA). Flash chromatography was performed according to the literature[10] procedure. Elemental analysis was performed by Atlantic Microlab, Inc., Atlanta, GA. Mass spectra were obtained from the Mass Spectrometry Facility, Cornell University, Ithaca, NY. Where analyses are indicated by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. THF was distilled from sodium ketyl.

9-Nor-9-phenyl-Δ^8-tetrahydrocannabinol (2b). PhLi (2.1 M in cyclohexane, 5.21 mmol) was added dropwise to a stirred solution of the ketone (1; 750 mg, 2.35 mmol)[5] in dry THF (75 ml) at −10 °C under N_2. After being stirred for 1 h at −10 °C, the reaction mixture was quenched with ice and extracted with several portions of ether. The combined extracts were washed with H_2O and brine, dried over $MgSO_4$, and concentrated to a pale yellow solid. It was stirred with $SOCl_2$ (309 mg, 2.6 mmol) and $ZnCl_2$ (8 mg, 0.07 mmol) in benzene (75 mL) for 4 h at ambient temperature. After being stirred with saturated aqueous $NaHCO_3$ for 15 min, the organic layer was washed with brine, dried ($MgSO_4$), and concentrated to a dark orange solid (780 mg). It was purified by preparative LC on a Waters 500A instrument (0.5% CH_3CN in isooctane) to give 301 mg (39%) of **2b** as an orange glass: NMR ($CDCl_3$) δ 0.87 (t, 3 H, ω-CH_3), 1.13 and 1.41 (s, 3 H, gem CH_3's), 6.10 (br s, 1 H, H-2), 6.12 (br s, 1 H, H-8), 6.30 (br s, 1 H, H-4), 7.2–7.5 (m, 5 H, aryl). Anal. ($C_{26}H_{32}O_2 \cdot {}^1/_4H_2O$) C, H.

9-Nor-9-*n*-butyl-Δ^8-tetrahydrocannabinol (2c). As described for the preparation of **2b**, *n*-butyllithium (2.4 M in hexane) was allowed to react with 1. Dehydration of the crude product (767 mg, 1.97 mmol) was effected by heating with *p*-toluenesulfonic acid (*p*-TSA; 500 mg) in benzene (25 ml) for 2 h. The cool reaction mixture was washed with H_2O and then several portions of saturated aqueous $NaHCO_3$, dried ($MgSO_4$), and concentrated to give an orange oil, which was purified by flash chromatography using 11% EtOAc in hexane. Compound **2c** was obtained (22% yield) as a yellow oil homogeneous by TLC (1:1 ether/hexane) and GC; NMR ($CDCl_3$) δ 0.87 (t, 3 H, ω-CH_3), 1.08 and 1.35 (s, 3 H, gem CH_3's), 1.9 (m, 2 H, H-11), 5.42 (br s, 1 H, H-8), 6.10 (br s, 1 H, H-2), 6.25 (br s, 1 H, H-4). M^+ calcd for $C_{24}H_{36}O_2$, 356.2715; found, 356.2725.

9-Nor-9-isopropyl-Δ^8-tetrahydrocannabinol (2d). The ketone 1 as the acetate[11] (750 mg, 2.37 mmol) in ether (10 mL) was added dropwise under N_2 to a stirred ethereal solution of $(CH_3)_2CHMgBr$[12] (0.78 M, 39.2 mmol). The reaction mixture was refluxed for 45 min and cooled, and the excess reagent was destroyed with CH_3OH. The mixture was neutralized with 3 N HCl, washed with H_2O, saturated $NaHCO_3$ solution, and brine, dried ($MgSO_4$), and concentrated to give a pale yellow solid. The material was dehydrated by refluxing with *p*-TSA as in **2c**. The material was purified by flash chromatography using 10% EtOAc in hexane (173 mg; 25% yield). GC analysis of the olefin (TMSE) showed two compounds present in a 9:1 ratio, which were separated by preparative GC. Subsequent desilylation of the major

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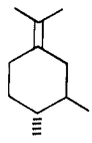
component, effected by heating with CH_3OH for 0.5 h, yielded **2d** as a yellow oil homogeneous by TLC (1:1 ether/hexane) and GC: NMR ($CDCl_3$) δ 0.87 (t, 3 H, ω-CH_3), 1.03 [d, J = 8.3 Hz, 6 H,, $CH(CH_3)_2$], 1.09 and 1.38 (s, 3 H, gem CH_3's), 5.48 (br s, 1 H, H-8), 6.11 (br s, 1 H, H-2), 6.31 (br s, 1 H, H-4). M^+ calcd for $C_{23}H_{34}O_2$, 342.2559; found, 342.2561.

The NMR of the enriched minor component showed broad singlets at δ 1.67 and 1.75 and the absence of the vinylic proton. This data are consistent with an exocyclic olefinic structure.



9-Nor-9-benzyl-Δ^8-tetrahydrocannabinol (2e). As detailed for **2d**, an ethereal solution of $C_6H_5CH_2MgBr$[13] (1.3 M, 32 mmol) was allowed to react with the acetate of the ketone **1** (1.0 g, 3.2 mmol). After workup and dehydration, the residue was fractionated by flash chromatography (9% EtOAc in hexane) to give a TLC-pure fraction (578 mg, 46% yield) of **2e** as a viscous yellow oil (>95% by GC): NMR ($CDCl_3$) δ 0.83 (t, 3 H, ω-CH_3), 1.07 and 1.36 (s, 3 H, gem CH_3's), 3.29 (br s, 2 H, benzyl at C-9), 5.48 (br s, 1 H, H-8), 6.05 (br s, 1 H, H-2), 6.28 (br s, 1 H, H-4), 7.25 (s, 5 H, aryl). M^+ calcd for $C_{27}H_{34}O_2$, 390.2559; found, 390.2548.

9-Nor-9-phenethyl-Δ^8-tetrahydrocannabinol (2f) was prepared the same way as **2c**, except that $C_6H_5(CH_2)_2MgBr$ was used. It was purified by flash chromatography (10% EtOAc in hexane) to give **2f** as an orange oil (42%) (>95% pure by GC): NMR ($CDCl_3$) δ 0.84 (t, 3 H, ω-CH_3), 1.05 and 1.33 (s, 3 H, gem CH_3's), 5.42 (br s, 1 H, H-8), 6.09 (br s, 1 H, H-2), 6.27 (br s, 1 H, H-4), 7.16 (s, 5 H, aryl). Anal. ($C_{28}H_{36}O_2$) C, H.

9-Nor-9-(1-hydroxypropyl)-Δ^8-tetrahydrocannabinol (2g). An ethereal solution of **3** (800 mg, 2.2 mmol)[6b] was added dropwise under N_2 to a stirred solution of C_2H_5MgBr (1.2 M in ether, 28 mmol). The reaction mixture was refluxed for 2 h, cooled, and decomposed with saturated NH_4Cl solution. The mixture was washed with H_2O and brine, dried ($MgSO_4$), and concentrated to give a residue, which was purified by flash chromatography (25% EtOAc in hexane). Compound **2g** was isolated (200 mg, 32% yield) as an orange glass homogeneous by TLC (1:1 EtOAc/hexane) and GC: NMR ($CDCl_3$) δ 0.89 (t, 6 H, ω-CH_3 and CH_3CH_2), 1.10 and 1.40 (s, 3 H, gem CH_3's), 4.08 (br t, J = 6 Hz, 1 H, $CHOH$), 5.77 (br s, 1 H, H-8), 6.17 (br s, 1 H, H-2), 6.32 (br s, 1 H, H-4). M^+ calcd for $C_{23}H_{34}O_3$, 358.2508; found, 358.2508.

9-Nor-9-(1-hydroxyethyl)-Δ^8-tetrahydrocannabinol (2h). In the manner described for **2g**, CH_3MgI was added to **3**,[6b] and **2h** was isolated by flash chromatography (30% EtOAc in hexane) in 55% yield. GC showed that it was a 1:1 mixture of two compounds with very close retention times (it is assumed to be a C-11 epimeric mixture): NMR ($CDCl_3$) δ 0.85 (t, 3 H, ω-CH_3), 1.02 and 1.32 (s, 3 H, gem CH_3's), 1.22 and 1.27 (partially observed d, CH_3CHOH), 4.21 (br q, J = 6 Hz, 1 H, $CHOH$), 5.65 (br s, 1 H, H-8), 6.09 (br s, 1 H, H-2), 6.20 (br s, 1 H, H-4). Anal. ($C_{22}H_{32}O_3 \cdot {}^1/_4H_2O$) C, H.

Pharmacology. The test drug (100 mg) was dissolved in 1 ml of a 1:1 mixture of emulphor (GAF Corp., Linden, NJ) and ethanol. With the aid of a sonicator, appropriate dilutions were made with the addition of emulphor/ethanol/saline (1:1:18).

Spontaneous Activity and Body Temperature in Mice. In order to conserve a limited supply of drug, we recorded rectal temperature and spontaneous activity in the same animal. Male ICR mice (22-30 g, obtained from Flow Laboratories, Dublin, VA) were housed in the laboratory for 24 h before treatment. The ambient temperature of the laboratory, which varies from 21 to 24 °C from day to day, was recorded at the beginning and end of each experiment. Rectal temperature was determined by a thermistor probe (inserted 25 mm) and a telethermometer (Yellow Springs Instrument Co., Yellow Springs, OH) just prior to vehicle or drug administration. Following the initial temperature determinations, mice were injected iv with either vehicle or drug (0.1 mL/10 g of body weight) and immediately placed individually in photocell activity chambers. Interruptions of the photocell beams were recorded for 10 min. The results are expressed as percent of control (vehicle treated), and the ED_{50}'s and their confidence limits were determined by the method of Litchfield and Wilcoxon.[14] The mice were removed from the activity chambers, and rectal temperatures were measured immediately and at 10-min intervals up to 60 min after drug administration. At least six mice were tested at each dose.

Antinociceptive Activity. The tail-flick procedure (time required for a mouse to flick his tail from a heat source) was carried out as previously described.[15] Control reaction time was determined in each mouse, and then vehicle or drug was administered iv into the tail vein and tested 5 min later. A maximum 10-s test latency was imposed if the animals did not respond. The percent maximum possible effect was calculated as follows:

$$\% \text{ max effect} = \frac{\text{test time} - \text{control reaction time}}{\text{cutoff time} - \text{control time}} \times 100$$

A minimum of six mice were tested at each dose. The phenylquinone-stretching test was performed as described by Pearl et al.[16] Test drug or vehicle was administered 1 min prior to the intraperitoneal administration of *p*-phenylquinone, and the number of stretches 5 and 10 min after the *p*-phenylquinone administration was averaged.

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Registry No. **1**, 52195-11-6; **2b**, 88703-57-5; **2c**, 88703-58-6; **2d**, 88703-59-7; **2e**, 88703-60-0; **2f**, 88703-61-1; **2g**, 88703-62-2; **2h** (isomer 1), 88703-63-3; **2h** (isomer 2), 88703-64-4; PhLi, 591-51-5; butyllithium, 109-72-8; isopropyl bromide, 75-26-3; benzyl bromide, 100-39-0; phenethyl bromide, 103-63-9; ethyl bromide, 74-96-4; methyl iodide, 74-88-4.

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