3′-Hydroxy- and (±)-3′,11-Dihydroxy-Δ^9-tetrahydrocannabinol: Biologically Active Metabolites of Δ^9-Tetrahydrocannabinol[1]

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Synthesis of 3′-hydroxy- (7) and (±)-3′,11-dihydroxy-Δ^9-tetrahydrocannabinol (11), metabolites of Δ^9-THC, is described. Condensation of the monoterpene (±)-*cis*-*p*-menth-2-ene-1,8-diol (1) and (±)-3′-acetoxyolivetol (2) in the presence of fused $ZnCl_2$ in CH_2Cl_2 gave a mixture from which the Δ^9-THC derivative **3**, containing small amounts of the Δ^8-isomer **4**, was isolated after column chromatography. This mixture was separated as their diacetates **5** and **6** by high-pressure liquid chromatography. Alkaline hydrolysis (5% KOH in MeOH) of **5** furnished the metabolite **7**. Condensation of (±)-**8** with **2** in the presence of *p*-toluenesulfonic acid gave **9a**, which was acetylated to **9b**. Treatment with $HgO/BF_3{\cdot}Et_2O$ in wet THF gave the aldehyde **10**. Reduction with $LiAlH_4$ furnished the metabolite **11**. These metabolites were compared with Δ^9-THC for their ability to depress spontaneous activity and rectal temperature in mice and for their effects on overt behavior in dogs. 3′-Hydroxy-Δ^9-THC was also compared to Δ^9-THC in the mouse sympatomatology test and cardiovascular system in dogs. The metabolites produced pharmacological effects similar to those of Δ^9-THC in all tests. 3′-Hydroxy-Δ^9-THC was 2–3 times more effective than Δ^9-THC in the behavioral tests, whereas (±)-3′,11-dihydroxy-Δ^9-THC was approximately 3 times less active than Δ^9-THC.

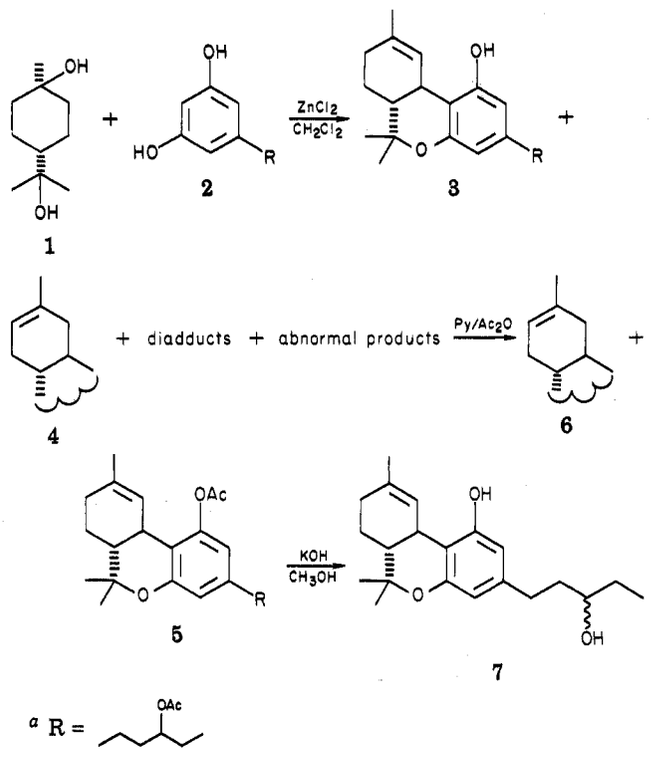
An understanding of the biotransformation of Δ^9-THC, the active constituent of marijuana, is of considerable importance in elucidating the mechanism of action of this drug in animals and man. Extensive literature has appeared in recent years describing the various metabolites of the cannabinoids isolated from in vivo or in vitro preparations.[2-4] These studies have been carried out on a wide variety of animal species, and in some cases different animal organ homogenates have been utilized. Important sites of metabolism of Δ^9-THC include oxidations at allylic positions, C-8 and C-11, in the terpene ring and/or at the aromatic *n*-pentyl side chain.[5] Some of these metabolites are pharmacologically equiactive with Δ^9-THC, and others are active to greater or lesser degrees. This has complicated the understanding of marijuana activity.

3′-Hydroxy-Δ^9-THC (7) is one of the two side-chain-hydroxylated metabolites of Δ^9-THC, isolated from the perfused dog-lung preparation by Widman et al.[6] Its formation was also reported in mouse liver preparations[7] and in cultures of *Cunninghamella blackesleeana*[8] and *Chaetomium globosum*.[9] In addition, the occurrence of 3′,11-dihydroxy-Δ^9-THC (11) has been reported for monkey liver preparations in vitro[4a] and in vivo mouse liver.[7]

In a preliminary communication[10a] we described the synthesis of 3′-hydroxy-Δ^9-THC (7, diastereoisomeric mixture at C-3′)[11] from a new synthon, (+)-*cis*-*p*-menth-2-ene-1,8-diol (1),[12] and reported it to be more potent than Δ^9-THC on the basis of overt symptomatology in mice.[10a] Wall and Brine[10b] reported similar activity for this metabolite. Independently of our work, Pitt et al.[13] have reported recently the synthesis of 3′-hydroxy-Δ^9-THC (7),[11] and Christie et al.[9] have described the synthesis of the totally racemic (±)-7.[11] Both authors used routes different from ours and did not report the pharmacological activity of this metabolite. In the present paper we present the details of our synthesis and confirm the biological activity and potency of this metabolite on the basis of overt behavior in dogs. It should be pointed out that the corresponding compounds in the Δ^8 series have been described.[14]

In addition to the monohydroxylated metabolite, we have synthesized the totally racemic (±)-3′,11-dihydroxylated metabolite (11) of Δ^9-THC and evaluated it for biological activity. To our knowledge, this dihydroxylated metabolite has not been synthesized and assessed for pharmacological and behavioral activity in either the Δ^8 or Δ^9 series.

Scheme I[a]



[a] R =

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(1) Paper 29 in the Hashish Series. For paper 28, see P. C. Meltzer, H. C. Dalzell, and R. K. Razdan, *Synthesis*, 985 (1981).
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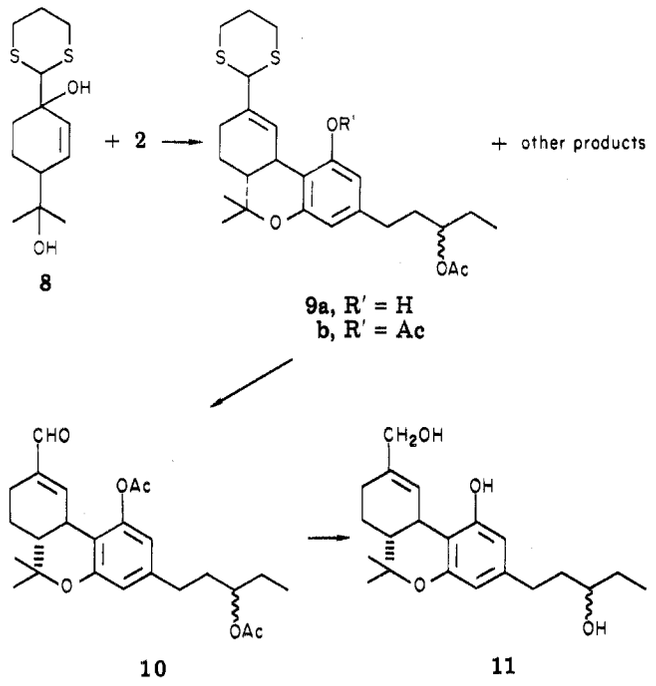
(3) R. Mechoulam, N. K. McCallum, and S. Burstein, *Chem. Rev.*, 76, 75 (1976).
(4) See, for example, (a) M. E. Wall and D. R. Brine, in "Marihuana: Chemistry, Biochemistry and Cellular Effects", G. G. Nahas, W. D. Paton, J. E. Idanpaan-Heikkila, Eds., Springer-Verlag, New York, 1976; (b) S. Burstein in "Cannabinoid Analysis in Physiological Fluids", J. A. Vinson, Ed., American Chemical Society, Washington, DC, 1979; *ACS Symp. Ser.*, **no. 98**.
(5) For a recent review, see S. Agurell, M. Binder, K. Foneska, J. E. Lindgren, K. Leander, B. Martin, J. M. Nilsson, M. Nordqvist, A. Ohlsson, and M. Widman in 4a, p 141.

0022-2623/82/1825-1447$01.25/0

Scheme II



Chemistry. In continuation of our search for various synthons[15] for the synthesis of Δ^9-THC and its metabolites, we have found that the readily available monoterpene (+)-*cis*-*p*-menth-2-ene-1,8-diol (**1**) can provide Δ^9-THC and that this synthon is particularly useful in the synthesis (Scheme I) of the metabolite 3′-hydroxy-Δ^9-THC (**7**), since only negligible amounts of iso-THC's were formed in the reaction mixture.

Thus, the isolation of **3** was considerably simplified. The condensation of **1** and (±)-3′-acetoxyolivetol (**2**)[16] was studied with a variety of catalysts (BF_3·Et_2O, *p*-toluenesulfonic acid, $ZnCl_2$, $ZnBr_2$, Dowex acid–ion-exchange resin, $SnCl_4$) and solvents (CH_2Cl_2, benzene, ether, etc.). The best conditions were found with fused $ZnCl_2$ in CH_2Cl_2 containing a small amount of THF. After column chromatography, the mixture of **3** and **4** was acetylated, and the Δ^9 and Δ^8 derivatives, **5** and **6**, respectively, were easily separated by high-pressure liquid chromatography (HPLC). The separation of the corresponding phenols **3** and **4** proved to be more difficult. Alkaline hydrolysis of **5** furnished the metabolite 3′-hydroxy-Δ^9-THC (**7**).

The synthesis of (±)-3′,11-dihydroxy-Δ^9-THC (**11**) was achieved (Scheme II) from the synthon **8** following essentially the procedure developed in our laboratory for the synthesis of various Δ^9-THC metabolites.[15d,17] Condensation of (±)-**8**[15d] with (±)-**2**[16] in refluxing C_6H_6 in the presence of *p*-toluenesulfonic acid formed a complex mixture from which **9a** was separated and was then acetylated to the diacetate **9b**. The dithiane masking group was hydrolyzed with mercury oxide and BF_3·Et_2O in wet THF to give the aldehyde **10**. Reduction of **10** with $LiAlH_4$ furnished the metabolite **11**.

Pharmacology and Discussion of Results. The synthetic metabolites were compared to Δ^9-THC in the mouse symptomatology test[18] and for their ability to alter spontaneous activity and body temperature in mice and blood pressure, heart rate, and overt behavior in dogs. This battery of tests allows for a more complete assessment of pharmacological activity than that provided by any one

Table I. Effects of Δ^9-THC and Its Metabolites on Spontaneous Activity and Rectal Temperature in Mice

dose, mg/kg	no. of mice	interruptions of the photocell/10 mins[a]	change in rectal temp,[a] °C
		Δ^9-THC	
vehicle	21	91 ± 5	+0.8 ± 0.1
2.5	6	59 ± 6	−2.6 ± 0.2
5.0	6	20 ± 8	−3.6 ± 0.5
7.5	8	17 ± 5	−3.8 ± 0.3
10.0	12	6 ± 3	−4.2 ± 0.6
		3′-Hydroxy-Δ^9-THC (7)	
vehicle	18	102 ± 10	+0.8 ± 0.1
0.1	12	108 ± 15	−0.4 ± 0.3
0.3	18	86 ± 16	−1.3 ± 0.3
1.0	12	27 ± 8	−2.8 ± 0.1
3.0	12	13 ± 7	−4.1 ± 0.4
10.0	6	7 ± 3	−7.6 ± 0.5
		3′,11-Dihydroxy-Δ^9-THC (11)	
vehicle	24	98 ± 9	0.9 ± 0.2
3	5	81 ± 7	−0.4 ± 0.4
6	6	59 ± 7	−0.2 ± 0.4
10	6	33 ± 5	−3.0 ± 0.4
30	6	26 ± 9	−4.3 ± 1.0

[a] Mean ± SE.

Table II. Maximum Behavioral Effects of Δ^9-THC and Its Metabolites on Overt Behavior in Dogs

drug	dose, mg/kg	no. of animals	av score
vehicle		4	0
Δ^9-THC	0.1	1	0
	0.2	4	3
	0.4	2	4
3′-hydroxy-Δ^9-THC (7)	0.05	2	1
	0.1	2	2
	0.2	2	4.5
3′,11-dihydroxy-Δ^9-THC (11)	0.2	1	1
	0.5	2	3

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(11) Our compound **7** has the absolute configuration 6a*R*, 10a*R*, and *RS* at C-3′. Christie et al.'s[9] compound (±)-**7** refers to the optically inactive isomer, which has 6a*RS*, 10a*RS*, and C-3′ *RS*.
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Table III. Cardiovascular Effects of 1 mg/kg Δ^9-THC and 3′-Hydroxy-Δ^9-THC in Anesthetized Dogs

drug	N	% change	
		blood pressure	heart rate
vehicle[a]	5	−5 (−1 to −10)	+17 (+20 to −3)
Δ^9-THC[b]	3	−46 (−42 to −49)	−39 (−30 to −45)
3′-hydroxy-Δ^9-THC (7)	2	−45 (−41 to −50)	−12 (−3 to −17)

[a] Produces transient effect on blood pressure and heart rate. [b] As previously reported by Martin et al.[21]

of the individual assays alone.

Mouse Symptomatology Test. This test was used initially to determine the dosage range of 3′-hydroxy-Δ^9-THC that was pharmacologically active. The minimal effective dose of 3′-hydroxy-Δ^9-THC (7) was found to be between 0.1 and 0.2 mg/kg (iv) and was thus more potent than Δ^9-THC (0.5 mg/kg in the same test).

Spontaneous Activity and Body Temperature in Mice. Both metabolites exhibited cannabinoid activity in mice by decreasing spontaneous activity and producing hypothermia in a dose-related manner (Table I). 3′-Hydroxy-Δ^9-THC (0.8, 0.4–1.5 mg/kg; ED_{50}, CL) was approximately 3 times more potent than Δ^9-THC (3.2, 1.7–6.2 mg/kg), whereas 3′,11-dihydroxy-Δ^9-THC (8.7, 3.6–20.8 mg/kg) was about 3 times less active than Δ^9-THC. Similar potency ratios were found for Δ^9-THC and its metabolites regarding their ability to lower rectal temperature. For example, mice receiving 2.5 mg/kg Δ^9-THC had body temperatures 3.4 °C lower than those of vehicle-treated mice, whereas mice treated with 1.0 mg/kg 3′-hydroxy-Δ^9-THC had a comparable decreasse (3.6 °C) in rectal temperature. Likewise, a 10 mg/kg dose of 3′,11-dihydroxy-Δ^9-THC decreased rectal temperature 3.9 °C as compared to vehicle-treated mice. The duration of hypothermia appeared the same for all three compounds.

Overt Behavior in Dogs. The data presented in Table II show that 3′-hydroxy-Δ^9-THC is almost twice as potent as Δ^9-THC and that 3′,11-dihydroxy-Δ^9-THC is 2 to 3 times less potent than Δ^9-THC in producing typical cannabinoid effects in mongrel dogs. The behavioral changes included hyperreflexia accompanied by marked static ataxia and sedation. 3′-Hydroxy-Δ^9-THC was identical with Δ^9-THC with regard to behavioral changes, as well as onset and duration of action. 3′,11-Dihydroxy-Δ^9-THC had a slightly quicker onset of action, and maximum effects were seen at 15 min, rather than 30 min as was observed for Δ^9-THC. Although 3′,11-dihydroxy-Δ^9-THC produced the full spectrum of cannabinoid effects, depression of spontaneous activity appeared to be predominate.

Cardiovascular Effects. The preliminary data presented in Table III show that at an intravenous dose in anesthetized dogs of 1 mg/kg 3′-hydroxy-Δ^9-THC produced a fall in blood pressure comparable to that seen with the same dose of Δ^9-THC. 3′-Hydroxy-Δ^9-THC differed from Δ^9-THC in that it had an insignificant effect on heart rate. The maximum effect of both compounds on blood pressure was seen at 30 min.

We conclude from these results that the metabolites produce pharmacological effects similar to those of Δ^9-THC. Hydroxylation at position 3′ appears to enhance the behavioral activity of Δ^9-THC 2–3 times, whereas dihydroxylation decreases activity to about one-third. It should be noted, however, that since the dihydroxylated metabolite 11 in the present study is totally racemic, the optically active levo isomer should be nearly equiactive with Δ^9-THC. Therefore, both metabolites could contribute significantly to the behavioral activity of Δ^9-THC.

Experimental Section

The NMR spectra were measured on a Varian T-60 spectrometer. The high-pressure liquid chromatographic separations were made with a Waters Associates ALC-202 chromatograph equipped with a Model 6000 solvent-delivery system. The analyses by gas chromatography were made on a Varian Aerograph Model 1440, equipped with a 6 ft × 0.125 in. i.d. stainless-steel column packed with 2% OV-17 on 100–200 mesh Supelcoport and a flame-ionization detector. Mass spectral data were provided by the Mass Spectrometry Facility at Cornell University, Ithaca, NY.

3′-Hydroxy-Δ^9-tetrahydrocannabinol (6a,10a-*trans*-3-Pentyl-6a,7,8,10a-tetrahydro-6*H*-dibenzo[*b*,*d*]pyran-1,3′-diol, 7). In a nitrogen atmosphere, a solution of 1.31 g (7.7 mmol) of (+)-*cis*-*p*-menth-2-ene-1,8-diol[12] (1) and 1.834 g (7.7 mmol) of 3′-acetoxyolivetol[16] (2) 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 during 1 h. The reaction temperature was 23 °C. 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 a rotary evaporator. The residue (2.83 g) was column chromatographed with 120 g of silica gel (230–400 mesh, Merck) and eluted with a 20:80 EtOAc/hexane mixture. The early eluted fractions contained the diadducts (649 mg; 21% yield; TLC, 30:70 EtOAc/hexane; R_f 0.62). Then came the "normal" monoadducts (3 and 4; 684 mg, 29% yield; R_f 0.43), and the "abnormal" monoadducts (263 mg, 11% yield; R_f 0.35). Unchanged 3′-acetoxyolivetol (2; 349 mg, 12% of the charge) was eluted with 1:1 EtOAc/hexane. The fractions containing the "normal" products were combined and consisted of a mixture of Δ^9 and Δ^8 isomers 3 and 4, 4:1 (GLC). This mixture of monoacetates 3 and 4 (635 mg, 1.7 mmol) was dissolved in 5.0 mL of pyridine, and 3.0 mL of Ac_2O was added. After the solution was left standing at room temperature for 3 h, a mixture of ether and ice–water was added, and the products were extracted into ether. The ether solution was washed with H_2O, 1 N HCl, followed by 5% $NaHCO_3$ and saturated brine. It was dried and concentrated to leave 664 mg (95%) of diacetates, which were separated by HPLC.

The best eluent was found to be a binary system of CH_3CN/isooctane, in a ratio of 2.5:98, which was homogeneous at about 30 °C. A stainless-steel column (7.8 mm × 122 cm) packed with Porasil C (Waters Associates) was used in a Waters 202 HPLC system. Recycling the injected sample (25 mg as a 30% solution in isooctane containing a trace of benzene to locate the solvent front) for five cycles, while shaving the sides of the peaks, separated the components well enough to allow recovery of suitably enriched Δ^9-diacetate 5, which eluted before the Δ^8-diacetate isomer 6 (elution factor k = 1.52 and 1.94, respectively). In this manner, a total of 192 mg of orange resin was recovered, which assayed >91% Δ^9-diacetate 5 (GLC: 2% OV-17, 280 °C, retention time 3.40 min; the GLC data for other compounds is Δ^8-diacetate isomer 6, 3.11 min; Δ^9-monoacetate 3, 4.59 min). An elution system could not be found that would work with the Δ^9- and Δ^8-monoacetates (3 and 4) in the same way.

A solution **Dithioacetal** 160 mg (0.385 mmol) of chromatographed Δ^9-diacetate 5 in 3 mL of CH_3OH was mixed with 4.3 mL of 5% KOH in CH_3OH (3.85 mmol of KOH) and allowed to stand at 50–55 °C. After 2 h the reaction was diluted with H_2O and extracted with ether. The ether layer was washed successively with saturated $NaHCO_3$, H_2O, and saturated brine and then dried and concentrated in a rotary evaporator. The residue was 117 mg (92%) of the diol 7, as a pale yellow resin of purity >91% (GLC): NMR (CCl_4) δ 6.35 (br s, 10-CH), 6.08 (s, 2 H, aryl), 3.48 (m, 3′-CH), 2.50 (m, 1′-CH_2), 1.65 (s, 9-C-CH_3), 1.31 and 1.02 [2 s, 6-C-$(CH_3)_2$], 0.88 (m, 5′-CH_3), 2 exchangeable OH; GLC (2% OV-17, column 280 °C) t_R 3.73 min; homogeneous by TLC (silica F-254, 1:4 EtOAc/hexane) R_f 0.06; mass spectrum, 330 (49), 315

(16), 258 (100), 247 (48). M^+ calcd for $C_{21}H_{30}O_3$, 330.2194; found, 330.2160. The overall isolated yield of the diol **7** was 7.4%.

(±)-3′-Acetoxy-11-oxo-Δ^9-tetrahydrocannabinol Trimethylene Dithioacetal 1-Acetate (9b). To a refluxing solution of (±)-3′-acetoxyolivetol (**2**; 981 mg, 4.12 mmol)[16] and *p*-toluenesulfonic acid (60 mg, 0.316 mmol) in 80 mL of C_6H_6 was added a solution of the terpene synthon (**8**; 801 mg, 2.92 mmol)[15d] in 10 mL of C_6H_6 and 5 mL of CH_2Cl_2. After 15 min, the reaction mixture was cooled, quenched with 20 mL of 5% NaOH, and diluted with 50 mL of ether. The organic layer was then washed with 25 mL of 5% aqueous NaOH, 25 mL of H_2O, and 25 mL of brine, dried ($MgSO_4$), and evaporated to a brown oil, which was purified by flash chromatography (silica gel 60, 230–400 mesh, 4:1 hexane/ethyl acetate) to **9a** as a light yellow oil: yield 94 mg (34%, based on 3′-acetoxyolivetol recovered from base wash); NMR (CCl_4) δ 0.85 (t, 3 H, ω-CH_3) 1.35 (s, 6 H, gem CH_3's), 1.98 (s, 3 H, 3′-OAc), 2.75 (br band, 6 H, SCH_2 and H-1′), 4.45 (s, 1 H, 2-dithianyl H), 4.78 (m, 2 H, H-3′ and OH), 6.03 (s, 2 H, H-2 and H-4), 6.88 (br s, H, H-10).

The phenol **9a** (94 mg 0.197 mmol) was dissolved in Ac_2O/Py (1:3, 8 mL) and left overnight. It was then poured into 50 mL of ice–water and extracted with ether. The combined organic layers were successively washed with 10% aqueous HCl saturated $NaHCO_3$ solution, and brine. It was dried ($MgSO_4$) and evaporated to give **9b** as a light yellow oil, homogeneous by TLC (92 mg, 100%): NMR (CCl_4) δ 1.95 (s, 3 H, 3′-OAc), 2.28 (s, 3 H, 1-OAc).

(±)-3′,11-Dihydroxy-Δ^9-tetrahydrocannabinol (11). To a dispersion of red HgO (81 mg, 0.37 mmol) in 5 mL of 15% H_2O/THF, under a N_2 atmosphere, were added BF_3·Et_2O (0.11 mL, 0.89 mmol) and a solution of **9b** (92 mg, 0.18 mmol) in 5 mL of THF. This was stirred for 3 h, quenched with 10 mL of 5% aqueous Na_2CO_3, and diluted with 50 mL of ether. The organic layer was separated and washed with 5% aqueous Na_2CO_3, H_2O, and brine, dried ($MgSO_4$), and evaporated to a pale yellow oil from which **10** was purified by preparative thin-layer chromatography (silica gel, 1:5 EtOAc/hexane) as a clear oil (36 mg, 47%): NMR (CCl_4) δ 0.88 (t, 3 H, ω-CH_3), 1.10 and 1.38 (s, 3 H, gem CH_3's), 1.95 (s, 3 H, 3′-OAc), 2.22 (s, 3 H, 1-OAc), 2.44 (d, 2 H, J = 8 Hz, H-1′), 3.37 (m, 1 H, H-10a), 4.77 (m, 1 H, H-3′), 6.35 (d, 1 H, J = 2 Hz, H-4), 6.45 (d, 1 H, J = 2 Hz, H-2), 7.20 (br s, 1 H, H-10), 9.33 (s, 1 H, CHO); IR ν_{max} (CCl_4) 1695, 1740, 1770 cm^{-1}.

The aldehyde (**10**; 36 mg, 0.084 mmol) was dissolved in 10 mL of THF and cooled in an ice bath, and $LiAlH_4$ (25 mg, 0.659 mmol) was added in small portions. It was stirred overnight and then quenched with 5 mL of saturated NH_4Cl. The mixture was filtered, and the solids were washed with ether (4 × 25 mL). The filtrates were evaporated to a clear oil, from which **11** was purified by prepartive thin-layer chromatography (silica gel, ether) as a clear gum, homogeneous by TLC (19 mg, 66%): NMR [$(CD_3)_2CO$] δ 0.90 (t, 3 H, ω-CH_3), 1.03 and 1.37 (s, 3 H, gem CH_3's), 2.78 (br s, 2 H, H-1′), 3.42 (br s, 1 H, H-3′), 3.93 (br s, 2 H, H-11), 6.15 (d, 1 H, J = 2 Hz, H-4), 6.30 (d, 1 H, J = 2 Hz, H-2), 6.75 (br s, 1 H, H-10); mass spectrum, *m/e* 346 (16), 315 (77), 259 (69), 241 (100), 199 (59). M^+ calcd for $C_{21}H_{30}O_4$, 346.2144; found, 346.2142.

The overall isolated yield of the metabolite **11** was 10.5%.

Pharmacology. The drugs were prepared in polyethylene glycol 400 for testing in the mouse symptomatology test. For all other tests, 100 mg of drug was dissolved in 1 mL of a 1:1 mixture of emulphor and ethanol, and appropriate dilutions were made with the addition of emulphor/ethanol/saline (1:1:18).

Mouse Symptomatology Test. Male albino CD-1 mice (18–22 g) were administered the drugs intravenously (iv) as a solution in polyethylene glycol 400 (0.06 mL/25 g of body weight). Various doses (0.1, 0.2, 1.0, and 3.0 mg/kg) of the test drug were given to at least three mice per dose to determine approximate minimum effective dose (MED's) for ataxia and sensitivity to touch ("popcorn"). These effects are characteristic of cannabinoids and have been described by us earlier.[18]

Spontaneous Activity and Body Temperature in Mice. In order to conserve a limited supply of drug, rectal temperature and spontaneous acitivity were recorded in the same animal. Male ICR mice (22–30 g) 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 termistor 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 intravenously with either vehicle or drug (0.1 mL/10 g of body weight) and immediately placed in photocell activity chambers. After the animals were placed in the chambers, interruptions of the photocell beams were recorded for 10 min. The results were expressed as percent of control, and the ED_{50}'s and their confidence limits were determined by the method of Litchfield and Wilcoxon.[19] 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.

Overt Behavior in Dogs. The ability of cannabinoids to produce static ataxia (an effect unique to psychoactive cannabinoids) and other characteristic behavioral effects was examined in mongrel dogs of either sex (8–12 kg). The animals were observed for their degree of spontaneous activity, gait, tail tuck, etc. prior to drug administration. The animals were then injected intravenously with the cannabinoid or vehicle (1 mL/5 kg of body weight), and their behavior was rated at 5-min intervals according to the Walton[20] static-ataxia scale as described recently.[21] A typical test session consisted of five animals that received either vehicle, 0.2 mg/kg Δ^9-THC, or one of three other cannabinoid treatments. Behavior was scored by three observers who are blind with regard to treatment.

Cardiovascular Studies. The experiments were conducted with mongrel dogs of either sex weighing between 6 and 11 kg, and responses were recorded on a Grass Model 5 polygraph. The dogs were anesthetized with sodium pentobarbital (30 mg/kg, iv), and the left femoral vein was cannulated to allow intravenous administration of drugs. Arterial pressure was recorded from the left femoral artery through a cannula connected to a Statham pressure transducer. Mean arterial pressure was calculated by summing the diastolic pressure and one-third of the difference between systolic and diastolic pressures. Heart rate was obtained from an EKG recording using fine needle electrodes inserted through the skin. Blood pressure and heart rate were recorded 1, 3, 5, 15, 30, 45, and 60 min after drug administration. The vehicle was administered to all animals before administration of the cannabinoid to ensure it was devoid of cardiovascular activity. Animals were given only one dose of one cannabinoid due to the drug's long duration of action. The maximum percent change in mean arterial blood pressure and heart rate (usually 30 min after the injection) was determined for each animal.

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