diluted with petroleum ether. The organic layer was washed successively with H_2O, 1 N $NaHCO_3$, and H_2O and dried over Na_2SO_4. The crude product was chromatographed on silica gel to give 3-(trimethylgermyl)acetophenone (**39**, 99%), which was condensed with methyl terephthalaldehydate in THF–*i*PrOH according to the method described for **21** to give **40** (22%). **40**: pale-yellow prisms (from AcOEt–*n*-hexane); mp 186–188 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 0.45 (s, 9 H), 7.50 (dd, 1 H, *J* = 7, 8 Hz), 7.62 (d, 1 H, *J* = 16 Hz), 7.72 (dt, 1 H, *J* = 1, 1, 7 Hz) 7.74 (d, 2 H, *J* = 8 Hz), 7.83 (d, 1 H, *J* = 16 Hz), 7.96 (ddd, 1 H, *J* = 1, 2, 8 Hz), 8.11 (m, 1 H), 8.14 (d, 2 H, *J* = 8 Hz). Anal. ($C_{19}H_{20}O_3Si \cdot {}^1/_4H_2O$) C, H.

(*E*)-4-[2-[3-(Trimethylsilyl)phenyl]ethenyl]benzoic Acid (45, St40S). A solution of *m*-bromotoluene (**41**, 7.7 g, 45 mmol) in 20 mL of dry ether was added slowly to 30% lithium dispersion in oil (2.32 g, 0.10 mol) in 10 mL of dry ether at −10 °C under Ar and the mixture was stirred for 3 h at room temperature. A solution of chlorotrimethylsilane (4.68 g, 43.1 mmol; freshly distilled from CaH_2) in 14 mL of dry ether was added, and the mixture was stirred for 36 h. After filtration, the filtrate was washed with H_2O and brine and dried over Na_2SO_4. The crude mixture was chromatographed on silica gel to give *m*-tolyltrimethylsilane (**42**, 54%). *N*-Bromosuccinimide (1.09 g, 6.12 mmol) and a catalytic amount of azobisisobutyronitrile were added to a solution of **42** (1.0 g, 6.09 mmol) in 6 mL of dry CH_2Cl_2 and the mixture was refluxed for 4.5 h. After cooling and filtration, the solvent was removed. The residue was chromatographed on silica gel to give 3-(trimethylsilyl)benzyl bromide (**43**, 70%). A mixture of **43** (730 mg, 3.00 mmol) and triphenylphosphine (1.18 g, 4.50 mmol) was refluxed in 10 mL of dry toluene under Ar for 4 h. [3-(Trimethylsilyl)benzyl]triphenylphosphonium bromide (**44**) was obtained by filtration (90%). Compound **44** (758 mg, 1.50 mmol) and methyl terephthalaldehydate (258 mg, 1.57 mmol) were added to a solution of Na (40 mg, 1.74 mmol) in 15 mL of dry methanol, and the mixture was stirred for 17 h. The precipitate was collected to give methyl (*E*)-4-[2-[3-(trimethylsilyl)phenyl]ethenyl]benzoate (St41S). The filtrate was chromatographed on silica gel to give St41S (total 70%) and its *Z* isomer (24%). St41S was hydrolyzed (aqueous KOH–EtOH) to give **45** (St40S, 85%). **St41S**: colorless needles (from CH_2Cl_2–*n*-hexane); mp 113–114.5 °C; ^1H NMR (100 MHz, $CDCl_3$) δ 0.30 (s, 9 H), 3.92 (s, 3 H), 7.0–7.6 (m, 8 H), 8.02 (d, 2 H, *J* = 8 Hz); UV λ_{max} (nm, log ε) 323 (4.91), 232 (4.61), 209 (4.75). **45 (St40S)**: colorless needles (from CH_2Cl_2–*n*-hexane); mp 216–218 °C; ^1H NMR (100 MHz, $CDCl_3$–DMSO-d_6) δ 0.30 (s, 9 H), 7.12 (d, 1 H, *J* = 18 Hz), 7.25 (d, 1 H, *J* = 18 Hz), 7.4–7.8 (m, 6 H), 7.95 (d, 2 H, *J* = 8 Hz). Anal. ($C_{18}H_{20}O_2Si \cdot {}^1/_4H_2O$) C, H.

Registry No. **3**, 541-73-1; **4**, 2060-89-1; **5**, 15290-24-1; **6**, 15290-25-2; **7**, 125973-48-0; **8**, 626-39-1; **9**, 5624-60-2; **10**, 78627-91-5; **11**, 125973-49-1; **12**, 125973-50-4; **13**, 125973-51-5; **14**, 125973-52-6; **15**, 125973-53-7; **16**, 125973-54-8; **17**, 81500-98-3; **18**, 125973-55-9; **19**, 125973-56-0; **20**, 17983-62-9; **21**, 125973-57-1; **22**, 125973-58-2; **23**, 99-90-1; **24**, 4360-68-3; **25**, 18192-28-4; **26**, 17983-61-8; **27**, 125973-59-3; **28**, 81805-70-1; **29**, 81805-71-2; **30**, 125973-60-6; **31**, 14401-73-1; **32**, 81500-93-8; **33**, 81500-96-1; **34**, 81500-99-4; **35**, 125973-61-7; **36**, 2142-63-4; **37**, 39172-32-2; **38**, 81805-70-1; **39**, 125973-62-8; **40**, 125973-63-9; **41**, 591-17-3; **42**, 3728-44-7; **43**, 17903-44-5; **44**, 125973-64-0; **45**, 125973-65-1; **46**, 102121-16-4; **47**, 116232-90-7; **48**, 104182-40-3; **49**, 119567-94-1; **50**, 110368-33-7; **51**, 119567-96-3; **52**, 102405-34-5; Am41S, 125973-66-2; Am56S, 125973-69-5; TMGCl, 1529-47-1; Am56G, 125973-70-8; Am556S, 125973-71-9; St41S (*E* isomer), 125973-72-0; St41S (*Z* isomer), 125973-73-1; *p*-$ClCOC_6H_4COOCH_3$, 7377-26-6; *p*-$NH_2C_6H_4COOH$, 619-45-4; *p*-$HCOC_6H_4COOCH_3$, 1571-08-0; 1-nitro-3,4-bis(trimethylsilyl)benzene, 78627-92-6; 1,3-dinitro-5-(trimethylsilyl)benzene, 125973-47-9; 3,3′,5,5′-tetrakis(trimethylsilyl)biphenyl, 125973-67-3; 2-nitro-1,3,5-tris(trimethylsilyl)benzene, 125973-68-4.

Synthesis and Pharmacological Evaluation of Amino, Azido, and Nitrogen Mustard Analogues of 10-Substituted Cannabidiol and 11- or 12-Substituted Δ^8-Tetrahydrocannabinol

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The synthesis of a variety of novel 10-substituted cannabidiol (CBD) and 11- or 12-substituted Δ^8-tetrahydrocannabinol (Δ^8-THC) analogues containing amino, alkylamino, azido, or a *N,N*-bis(2-chloroethyl)amino functional group is described, as well as their pharmacological evaluation in mice. These analogues, which possess only a portion of the full pharmacological spectrum of activity of Δ^9-THC, indicate that cannabinoid-mediated reduction of spontaneous locomotor activity, hypothermia, antinociception, and/or catalepsy need not be produced simultaneously, possibly suggesting the existence of more than one mechanism of action. The 10-substituted CBD analogues **3**, **4**, and **5** with an ethylamino, propylamino, or azido functional group, respectively, proved to be largely inactive, except for the production of central nervous system (CNS) depression concomitant with toxicity. Toxicity and CNS depression may be related phenomena in these nitrogenous compounds since 12-amino and 12-ethylamino analogues (**8** and **11**) of Δ^8-THC also proved to be very toxic. Antinociceptive and hypothermic responses (without reduction of motor activity) were observed at a dose of 10 mg/kg of the 11-ethylamino analogue (**9**) of Δ^8-THC, while a dose of 50 mg/kg of the nitrogen mustard 11-[*N,N*-bis(2-chloroethyl)amino]-Δ^8-THC (**12**) was necessary to produce any observable pharmacological effect. When selected analogues were evaluated for antagonistic properties, they failed to attenuate the effects of Δ^9-THC. Some nitrogen mustard analogues were capable of producing minimal pharmacological effects after either peripheral or direct CNS administration; however, these analogues also failed to attenuate the effects of Δ^9-THC either immediately after administration or 24–48 h later.

Δ^9-Tetrahydrocannabinol (Δ^9-THC) and certain analogues (e.g. Δ^8-THC) produce characteristic psychotropic responses in humans, as well as specific behavioral alterations in laboratory animals.[1] Other naturally occurring cannabinoids, such as cannabidiol (CBD, **1**), have been found to be devoid of many of these pharmacological effects, as well as devoid of psychoactive properties.[2] The pharmacological effects of the cannabinoids have been

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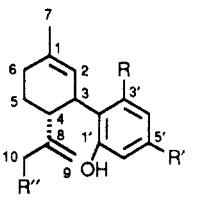
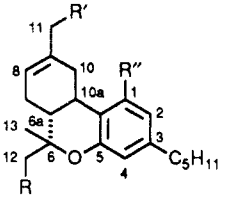
Table I. Derivatives of Cannabidiol and Δ^8-THC



1–6



7–12

CBD analogues				Δ^8-THC analogues			
	R	R′	R″		R	R′	R″
1 (CBD)	OH	C_5H_{11}	H	7(Δ^8-THC)	H	H	OH
2	C_5H_{11}	OH	H	8	NH_2	H	OH
3	OH	C_5H_{11}	$NH(C_2H_5)$	9·HCl	H	$NH(C_2H_5)$	OH
4·HCl	OH	C_5H_{11}	$NH(C_3H_7)$	10·HCl	H	$N(C_2H_4Cl)_2$	OAc
5	OH	C_5H_{11}	N_3	11	$NH(C_2H_5)$	H	OH
6	OH	C_5H_{11}	$N(C_2H_4Cl)_2$	12	H	$N(C_2H_4Cl)_2$	OH

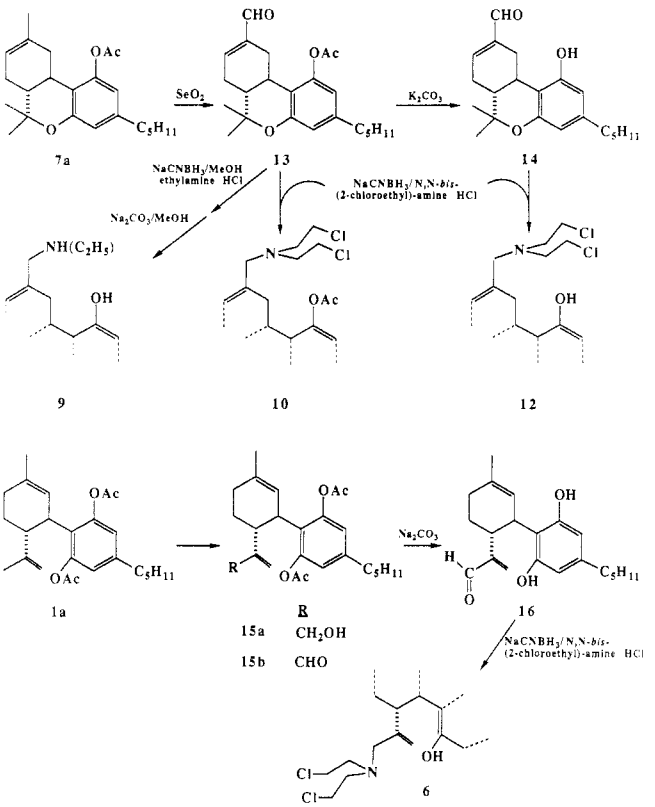
hypothesized to be mediated by a variety of mechanisms; however, no direct-acting cannabinoid antagonist exists to further molecular investigations into these possible mechanisms of action. Another well-recognized aspect of cannabinoid chemistry is that this drug class does not contain a nitrogen atom, making nitrogen containing functional group addition obvious for further investigation of cannabinoid structure–activity relationships (SAR).

Modification of CBD can lead to analogues with unique pharmacological profiles. Abnormal CBD (ABN-CBD, **2**) (where the phenolic hydroxy group has been transposed with the 5-carbon side chain) does not produce overt behavioral effects in the dog[3] or mouse,[4] but does produce potent alterations in the dog cardiovascular system.[3] However, this analogue has not been tested for possible antagonistic properties. Similarly, another CBD analogue which possesses selective activity is 10-(*N*-ethylamino)-CBD (**3**) (whose synthesis from 10-bromo-CBD diacetate has previously been reported[5a]). 10-(*N*-Ethylamino)-CBD selectively depresses spontaneous activity in mice, without producing significant hypothermia or antinociception.[5a] However, 10-(*N*-ethylamino)-CBD has been evaluated for antagonistic properties and was reported to partially attenaute the antinociceptive actions of Δ^9-THC.[5a]

Therefore, in continuation of previous work,[5] a novel series of nitrogen-containing derivatives of Δ^8-THC and CBD (Table I) was synthesized and evaluated in various pharmacological paradigms to further assess cannabinoid SAR and to determine possible antagonistic properties. Analogues of CBD (**1**) were named by using the monoterpenoid numbering system, while analogues of Δ^8-THC (**7**) were designated according to the dibenzopyran numbering system.

Chemistry. We previously reported a practical synthesis of ABN-CBD (**2**)[8,9] and of 10-(*N*-ethylamino)-CBD

Scheme I



(**3**) from 10-bromo-CBD diacetate.[5] We have now prepared 10-(*N*-propylamino)-CBD analogue **4**. Treatment of 10-

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Table II. Dose–Response Data (Mean ± SEM) for Cannabinoid Analogues[c]

compd	dose, mg/kg	spont activity, counts/10 min	temp, °C	tail-flick latency, s	catalepsy (% immobility)
2	0	45 ± 6	−1.4 ± 0.3	0.2 ± 0.2	4 ± 1
	100	57 ± 9	−3.5 ± 0.2*	0.8 ± 0.3	30 ± 2*
3	0	53 ± 9	−0.8 ± 0.2	0.6 ± 0.2	4 ± 1
	3	27 ± 5*	−2.1 ± 0.3*	1.0 ± 0.4	7 ± 2
6	0[b]	90 ± 18	−1.1 ± 0.3	−0.2 ± 0.2	NT[a]
	100[b]	81 ± 23	−1.8 ± 0.4	0.8 ± 0.4	NT
	150[b]	75 ± 25	−3.8 ± 0.8*	1.5 ± 0.4*	NT
8	0	68 ± 9	−0.8 ± 0.2	0.6 ± 0.1	13 ± 3
	3	43 ± 5*	−1.1 ± 0.3	1.1 ± 0.2	24 ± 5
	5	42 ± 6*	−0.7 ± 0.3	1.9 ± 0.4*	15 ± 3
	6	25 ± 4*	−3.0 ± 0.4*	1.1 ± 0.2	NT
9	0	49 ± 7	−1.8 ± 0.3	0.5 ± 0.3	NT
	10	54 ± 11	−3.8 ± 0.8*	2.6 ± 0.5*	NT
10	0	51 ± 12	−1.8 ± 0.5	0.5 ± 0.3	4 ± 2
	50	26 ± 10	−3.3 ± 0.5*	0.6 ± 0.5	31 ± 4*
11	0	52 ± 11	−0.9 ± 0.3	1.0 ± 0.1	13 ± 3
	3	35 ± 7	−1.5 ± 0.3	2.8 ± 0.6*	20 ± 4
	5	35 ± 5	−0.7 ± 0.3	2.2 ± 0.5	16 ± 5
12	0	56 ± 7	−0.8 ± 0.3	0.7 ± 0.3	4 ± 2
	50	27 ± 8*	−3.8 ± 0.5*	−0.6 ± 0.3	24 ± 5*
	0[b]	87 ± 9	−1.5 ± 0.3	0.2 ± 0.2	NT
	150[b]	70 ± 11	−1.7 ± 0.2	0.0 ± 0.2	NT
Δ^9-THC	0	54 ± 5	−1.1 ± 0.2	0.6 ± 0.2	8 ± 1
	0.3	70 ± 7	−1.0 ± 0.2	2.0 ± 0.4*	9 ± 3
	3	30 ± 3*	−2.4 ± 0.2*	2.9 ± 0.5*	25 ± 3*
	6	23 ± 5*	−2.9 ± 0.2*	4.9 ± 0.5*	42 ± 3*
	30	7 ± 1*	−5.1 ± 0.3*	5.1 ± 0.4*	57 ± 3*
	0[b]	103 ± 26	−2.2 ± 0.6	−0.2 ± 0.3	NT
	50[b]	46 ± 12	−2.3 ± 0.6	1.6 ± 0.4*	NT
	100[b]	41 ± 15*	−3.6 ± 0.6	2.3 ± 0.6*	NT
	150[b]	33 ± 13*	−5.5 ± 0.4*	4.0 ± 0.6*	NT

[a] NT indicates not tested. [b] Dose represents total micrograms administered intraventricularly. [c] An asterisk denotes values where $p < 0.05$ versus control.

bromo-CBD diacetate with *n*-propylamine in DMSO followed by alkaline hydrolysis (Na_2CO_3) gave **4** in 33% yield. Azido compound **5** was obtained in 70% yield from 10-azido-CBD diacetate[5a] by using the same hydrolytic conditions as in the synthesis of **4**. 12-Amino-Δ^8-THC derivative **8** was synthesized from 12-hydroxy-Δ^8-THC.[5b] Thus, the 12-hydroxy group was first converted to the 12-azido group via its triflate and then reduced with $LiAlH_4$ to give compound **8**. The overall yield was low (16%) primarily because the azido compound was obtained in poor yield (20%). For the preparation of compound **11** (the 12-(*N*-ethylamino) analogue of Δ^8-THC), the nitrogen in **8** was selectively acetylated by using tetraacetylglycoluril[19] in CH_3CN and then reduced with $LiAlH_4$ (overall yield 45%). 11-(*N*-Ethylamino)-Δ^8-THF (**9**, free base) and the mustard analogues **10** and **12** (Scheme I) were all prepared from 11-oxo-Δ^8-THC acetate (**13**).[18] The latter was obtained by SeO_2 oxidation of Δ^8-THC acetate (**7a**) in 20% isolated yield. The reductive amination of **13** with $NaCNBH_3$[20] and ethylamine hydrochloride in methanol containing 3-Å activated molecular sieves afforded **9** as the *O*-acetate, which was hydrolyzed with Na_2CO_3/MeOH. The desired amine (49%) was isolated as the hydrochloride salt **9**. For the synthesis of the mustard analogues **10** and **12**, the best procedure found was to carry the reductive amination of the aldehyde in the presence of $NaCNBH_3$ and *N*,*N*-bis(2-chloroethyl)amine hydrochloride as in **9**. All attempts to prepare the mustards by using other procedures (such as reductive amination with diethanolamine followed by either thionyl chloride or by using the procedures of Wiley et al.[21] and Portoghese et al.[22]) failed under a variety of conditions. The mustard analogues, not surprisingly, appear very prone to degradation and all attempts to hydrolyze the acetyl group of mustard **10** to form **12** failed under a variety of conditions. However, aldehyde **13** was hydrolyzed easily to **14** in 90% yield which, when subjected to reductive amination conditions, formed the desired mustard analogue **12** (27%). In view of this, we used a similar approach for the synthesis of mustard analogue **6** in the CBD series. Thus, 10-hydroxy-CBD diacetate **15a** [obtained from CBD diacetate (**1a**)[5b]] was oxidized with pyridinium chlorochromate to give aldehyde **15b**, which was then hydrolyzed to **16** and converted to the mustard analogue **6** (Scheme I). It should be noted that in an alternative synthesis[25] **15b** was selectively obtained by the oxidation of CBD diacetate (**1a**) with SeO_2.

Pharmacology and Discussion of Results

The structures of compounds **1**–**12** are found in Table I, and results obtained with these analogues, as well as Δ^9-THC, are given in Table II. The variability of the vehicle control responses observed with each of the independent evaluations of the novel compounds was found to be greatest with the spontaneous activity measurements, very moderate in the immobility rating, and quite small

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with both temperature and tail-flick latency values. With the exception of intraventricular administration, control temperature changes varied no more than 1 °C (0.8–1.8 °C) and control latencies varied less than 1 s (0.2–1.0 s). The range of control catalepsy values was only 4–13%. However, control spontaneous activity values varied considerably (45–68 counts) across independent sets of experiments, which resulted from the variability in animal response.

Δ^9-THC is active in the antinociceptive test at doses as low as 0.3 mg/kg, produces an effect in all tests at 3 mg/kg, and is maximally active at 30 mg/kg in depressing spontaneous activity and producing hypothermia. However, the tail-flick latency and catalepsy induced by a dose of 30 mg/kg Δ^9-THC are not significantly different (ANOVA) from the effect produced by 6 mg/kg Δ^9-THC. Intraventricular administration of Δ^9-THC produced behavioral effects in all tests at doses of 50–150 μg.

Agonistic Activity of CBD Analogues. Administration of 3–30 mg/kg ABN-CBD (**2**) failed to produce any effects, while 100 mg/kg **2** produced a decrease in temperature (2.1 °C greater than control) and a moderate degree of catalepsy (approximately equal to that found with 3 mg/kg Δ^9-THC). Thus, **2** was approximately 20-fold less potent than Δ^9-THC in the production of hypothermia and catalepsy. As previously observed,[5] 10-(*N*-ethylamino)-CBD (**3**) decreased spontaneous activity at 3 mg/kg, yet did not produce antinociception or catalepsy. However, unlike the previous report,[5a] production of hypothermia was observed. Higher doses could not be tested to determine if these were dose-dependent responses since there was 100% mortality at 10 mg/kg.

Administration of 1–10 mg/kg **4** did not produce any pharmacological effects; therefore, data are not included in Table II. Deaths occurred at 30 mg/kg **4**, with 100% mortality at 100 mg/kg. It appears that an increase of only one carbon in the length of the 10-alkylamino group is sufficient to reduce toxicity by as much as 10-fold. However, the lack of cannabimimetic activity of **4** may suggest that the depression and hypothermia produced by **3** are due to general central nervous system (CNS) depression, rather than actions through specific mechanisms. Administration of 1–10 mg/kg **5**, the azido derivative of CBD, also failed to produce significant pharmacological effects; therefore, data are not included in Table II. Larger doses were not evaluated.

The intraventricular route of administration was chosen for **6**, since it is possible for a nitrogen mustard to alkylate peripheral tissue and not reach the CNS. Intraventricular administration of **6** failed to produce any effects except at the 150-μg dose (Table II), which significantly reduced temperature and increased tail-flick latency. Comparison to Δ^9-THC suggests that **6** is approximately $^2/_3$ as potent as Δ^9-THC in the production of hypothermia, but approximately $^1/_3$ as potent in the production of antinociception. Thus, nitrogen mustard analogues are capable of producing pharmacological effects within the CNS.

Agonistic Activity of Δ^8-THC Analogues. Potent CNS depression was observed for **8** (Table II). Significant depression of spontaneous activity occurred at a dose as low as 3 mg/kg, while a 63% reduction was observed at a dose of 6 mg/kg of **8**. A similar reduction of activity was observed after 6 mg/kg Δ^9-THC, suggesting similar potencies for these compounds. A dose of 6 mg/kg of **8** also produced a decrease in temperature of 2.2 °C greater than vehicle control, which is also similar to the decrease of 1.8 °C observed after 6 mg/kg Δ^9-THC, further suggesting similar potency. Production of antinociception by **8** at 5 mg/kg was not considered significant since no other dose produced this effect. Analogue **8** also failed to produce catalepsy at 1–5 mg/kg. Unfortunately, higher doses could not be evaluated because deaths occurred at 8 and 10 mg/kg. Thus, it appears possible to produce potent CNS depression without producing antinociception or catalepsy. These data suggest that the mechanisms involved in the production of antinociception and/or catalepsy are distinct from those involved in the production of hypothermia and/or hypoactivity. Additionally, **9** at a dose of 10 mg/kg produced hypothermia and moderate antinociception. These data, combined with those presented for other analogues, suggest that hypoactivity, hypothermia, and antinociception may occur independently of one another and thus may be produced by different mechanisms of action. Lastly, the toxicity and the spectrum of pharmacological activity of **8** are similar to those of the CBD analogue **3**. Thus, it is possible that hypothermia, hypoactivity, and the toxicity observed with alkylamino cannabinoids are related phenomena which are produced by different degrees of general CNS depression. Regardless, it appears that the addition of amino and alkylamino functional groups to cannabinoids increases toxicity. Further support for this contention is the fact that **9** (an ethylamino analogue of Δ^8-THC) also produced death at 20 mg/kg. Compound **11**, another 12-substituted alkylamino, was not evaluated at doses larger than 5 mg/kg; thus, acute toxicity is unknown. However, **11** does not produce significant pharmacological activity.

The nitrogen mustard analogues **10** and **12** were inactive in the dose range of 1–30 mg/kg. However, a dose of 50 mg/kg of **10** and **12** reduced spontaneous activity by 49% and 52%, respectively, which makes each approximately 10-fold less potent than Δ^9-THC. Both **10** and **12** also produced hypothermia but did not produce antinociception. The immobility produced by **10** and **12** were quantitatively similar to that produced by Δ^9-THC at 3 mg/kg, indicating that these analogues are approximately 15-fold less potent. Thus, it is understandable that 150 μg of intraventricularly administered **12** is inactive (Table II). These data further suggest that the nitrogen mustards are largely inactive due to their structural modifications, rather than because of their presumed inability to reach the brain.

Antagonistic Activity of CBD and Δ^8-THC Analogues. Except for **11**, all novel compounds, as well as 10-bromo-CBD diacetate and 10-(*N*-ethylamino)-CBD (**3**), were evaluated for their ability to attenuate the effects of 6 mg/kg Δ^9-THC (data not shown). In each series of experiments Δ^9-THC alone always produced a significant effect. The dose of various cannabinoid analogues evaluated for antagonistic properties were (a) the largest dose which produced no effect in evaluations for agonistic activity or (b) the minimally active dose of an analogue which possessed agonistic activity in only one of the four behavioral evaluations. Results indicate that none of the compounds produced a significant alteration in any of the four Δ^9-THC-mediated behaviors. Additionally, antagonism by the nitrogen mustards **10** and **12** at 24 and 48 h posttreatment was not observed. The only exception is that **2** significantly (ANOVA, $p < 0.05$) increased catalepsy from 29% (±4) for Δ^9-THC alone to 52% (±5) for the combination treatment (data not shown). Additionally, 10-(*N*-ethylamino)-CBD (**3**) did not attenuate the antinociceptive effect of Δ^9-THC as reported previously.[5] The reasons for this discrepancy may be related to the different doses of Δ^9-THC used in these two studies. In the present report, antagonism was evaluated with a dose of Δ^9-THC which produced a maximal latency reponse, rather than

a dose which produced a response approximately 50% of maximum. Thus, possible antagonism by 10-(*N*-ethylamino)-CBD may not have biological significance.

Conclusions

Toxicity to Δ^9-THC appears to generally be due to respiratory depression with associated congestive, hemorrhagic, and edematous lung tissue, with reported mouse LD_{50} values for intravenous Δ^8-THC and Δ^9-THC being approximately 30 and 60 mg/kg, respectively.[14,15] The fact that many of these novel nitrogenous analogues produced death at 10–20 mg/kg suggests that amino and alkylamino functional groups enhance general CNS depression. However, this enhancement was not necessarily extended to the nontoxic cannabimimetic effects. In fact, most of these novel compounds were inactive, or only produced a limited range of effects and often did so at doses many times higher than that of Δ^9-THC. The fact that other cannabinoids with similar substituents were also inactive and very toxic[2] supports the contention that amino or alkylamino substituents generally produce inactive cannabinoids of extreme toxicity. Though toxicity produced by 60 mg/kg Δ^9-THC can probably be attributed to either general CNS depression or nonspecific molecular mechanisms, it is still possible that the lethality of the these novel nitrogenous cannabinoids might be due to mechanisms other than those of Δ^9-THC-mediated toxicity.

Only 10-(*N*-ethylamino)-CBD (**3**) and **8** proved to be of similar potency as Δ^9-THC, though only in depression of spontaneous locomotor activity and production of hypothermia. It is possible that these effects of **3** and **8** are related to the same general CNS depression responsible for toxicity. However, this contention clearly cannot be extended to Δ^9-THC itself. Thus, it is possible that the behavioral and toxic effects of these nitrogen-containing cannabinoids are also produced by distinctly different mechanisms of action and simply occur at relatively similar doses. Nevertheless, data suggest that depression of spontaneous activity, hypothermia, antinociception and/or catalepsy may be produced independently, possibly suggesting multiple mechanisms of action. However, complete dose responsiveness and testing of a wide range of doses to verify this assumption could not be performed.

Many of these novel analogues are at least 10–20-fold less potent than Δ^9-THC. Thus, it seems likely that behavioral effects produced by 50–100 mg/kg of cannabinoids are not biologically significant and that the analogues probably could not be used to probe for specific molecular mechanisms of action. The extreme lipophilicity of Δ^9-THC may allow it to partition into biological membranes and perturb their structure in a fashion similar to general anesthetics.[16,17] Therefore, it seems likely that the moderate degrees of catalepsy produced by 50–100 mg/kg cannabinoids are due to nonspecific actions (e.g. membrane disruption), rather than by the (presumably) specific mechanisms through which Δ^9-THC produces a similar response at doses less than 10 mg/kg.

Specific mechanisms through which cannabinoids may be producing biological effects include a binding site recently defined[23] with a potent cannabinoid analogue.[12] It has been hypothesized that this receptor is responsible for mediating the antinociceptive effects produced by Δ^9-THC.[23] Others have also suggested the existence of a specific cannabinoid site, though possible function was not clearly defined.[24] Thus, it should be possible to develop specific receptor antagonists (if one or more THC receptor exists) from inactive cannabinoid analogues. Another method of developing an antagonist is to synthesize a nitrogen mustard derivative of an active analogue, which might then function as a site-directed alkylator (i.e. receptor probe). Development of an irreversible antagonist using this approach has been successful in other areas of receptor research (e.g. adrenergic, opiate, and phencyclidine). However, previous attempts to develop an antagonist of Δ^9-THC by synthesizing a carbamate analogue proved unsuccessful.[6] Failure may have been due to the fact that the chemical modification in the carbamate derivative blocked the normally unhindered phenolic hydroxy group, which is considered necessary for cannabimimetic activity.[4,7] We therefore decided to modify our approach by synthesizing derivatives carrying a mustard moiety while retaining the free phenolic hydroxyl group. Altering the position of the mustard group allows further exploration of a different receptor locus of the nucleophile. Synthesis of analogues meeting these criteria were accomplished in both the Δ^8-THC series (**10** and **12**) and in the nonpsychoactive CBD series (**9**). Unfortunately, these analogues do not antagonize the acute pharmacological effects of Δ^9-THC. The possibility of a delayed pharmacological blockade by these mustards after either a 24- or 48-h pretreatment period is untenable. Despite the fact that many of the other nitrogenous cannabinoids are devoid of activity, none significantly attenuate Δ^9-THC-mediated effects. The failure to find an analogue capable of even partially attenuating the pharmacological effects of Δ^9-THC may indicate another possibility. Perhaps it is impossible to develop a specific THC-receptor antagonist because a specific site through which Δ^9-THC mediates its actions does not exist. Alternatively, a specific site may exist, but it is one to which none of the inactive analogs can interact and thus cannot prevent site occupation by cannabinoid agonists or attenuate the effects of Δ^9-THC. However, there is still reason to believe that a receptor antagonist may be synthesized, since the cataleptogenic effect of Δ^9-THC has been reported to be attenuated by its acid metabolite (9-nor-9-carboxy-Δ^9-THC).[26]

Experimental Section

Chemistry. The infrared spectra were recorded on a Perkin-Elmer Model 1320 spectrophotometer and the NMR spectra were measured on a Varian T-60 spectrometer with tetramethylsilane as an internal standard. 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.

Compounds **2** (ABN-CBD) and **3** [10-(ethylamino)-CBD] were prepared according to our previously reported procedures.[5,8,9]

10-(*N*-Propylamino)cannabidiol Hydrochloride (4). To a stirred solution of 530 mg (1.2 mmol) of 10-bromocannabidiol diacetate[5] in 30 mL of DMSO was added 0.2 mL (2.4 mmol) of *n*-propylamine at room temperature. After stirring for 1 h, the reaction mixture was poured into 150 mL of H_2O and extracted with ethyl acetate (3 × 100 mL). The combined ethyl acetate extracts were dried (Na_2SO_4) and concentrated in vacuo to give a brown oil which was purified by flash chromatography (silica gel; 5% MeOH/CH_2Cl_2) to give 255 mg (47%) of 10-(*N*-propylamino)cannabidiol diacetate (**4a**) as a colorless oil: ^1H NMR ($CDCl_3$) δ 0.86 (t, 3 H, ω-CH_3), 1.66 (s, 3 H, H-7), 2.23 (s, 6 H, OAc), 3.46 (br s, 2 H, H-10), 4.76 (br s, 1 H, H-3), 4.83 (br s, 2 H, H-9), 5.23 (br s, 1 H, H-2), 6.70 (s, 2 H, Ar H); IR ν_{max} (film) 2900, 1740 cm^{-1}.

To a stirred solution of 255 mg (0.56 mmol) of **4a** in 15 mL of MeOH was added a solution of 455 mg (4.29 mmol) of Na_2CO_3 in 1.5 mL of H_2O. Both solutions were deoxygenated by N_2 bubbling before mixing. After stirring for 2.5 h at room temperature, the reaction mixture was acidified with 1 N HCl, poured

(26) Burstein, S.; Hunter, S. A.; Latham, V.; Renzulli, L. *Experientia* **1987**, *43*, 402.

into 150 mL of brine, and extracted with ether (5 × 80 mL). The combined ether extracts were dried (Na_2SO_4) and concentrated in vacuo. The residual brown gum was purified by flash chromatography (silica gel; 5% MeOH/CH_2Cl_2) to give 148 mg (71%) of **4** as the free base: IR ν_{max} (film) 3400, 2900, 1560, 1410 cm^{-1}.

Hydrochloride salt **4** was obtained by treatment of the free base in dry ether with excess ethereal HCl solution. The solvent was removed in vacuo and the residue was twice reprecipitated from CH_2Cl_2 solution with dry ether. After drying in vacuo at room temperature, 35 mg (21%) of **4** was obtained as a cream solid: mp 150–155 °C (very hygroscopic); homogeneous by TLC (10% MeOH/$CHCl_3$). Anal. ($C_{24}H_{38}ClNO_2$) C, H, N, Cl.

10-Azidocannabidiol (5). The acetate groups of 10-azidocannabidiol diacetate, prepared according to our reported procedure,[5] were hydrolyzed by following the same procedure as described above for **4**. Azide **5** was purified by flash chromatography (silica gel; 5% MeOH/CH_2Cl_2) and was obtained in 70% yield as a gum. Anal. ($C_{21}H_{29}N_3O_2$) C, H, N.

10-Oxocannabidiol Diacetate (15b).[25] To a stirred suspension of 0.20 g (1.80 mmol) of anhydrous sodium acetate and 1.04 g (4.8 mmol) of pyridinium chlorochromate in 60 mL of dry CH_2Cl_2 at 0 °C was added (dropwise over 10 min) a solution of 1.00 g (2.40 mmol) of **15a**[5b] in 20 mL of CH_2Cl_2. After stirring for 1 h at 0 °C and 1 h at room temperature, the reaction mixture was filtered through a pad of Florisil (silica gel; Fisher Prod.) and washed with 4% MeOH/CH_2Cl_2. The filtrate was concentrated in vacuo to give a yellow gum, which was purified by flash chromatography (silica gel; 10% ethyl acetate/petroleum ether) to give 0.48 g (48%) of **15b** as a colorless oil: ^1H NMR ($CDCl_3$) δ 0.86 (t, 3 H, ω-CH_3), 1.70 (s, 3 H, H-7), 2.11 (s, 6 H, OAc), 3.80 (m, 1 H, H-3), 5.20 (s, 1 H, H-2), 5.80 (s, 1 H, H-9), 6.07 (s, 1 H, H-9), 6.66 (s, 2 H, Ar H), 9.33 (s, 1 H, H-10); IR ν_{max} (film) 1740, 1670 cm^{-1}.

10-Oxocannabidiol (16). A solution of 0.37 g (0.89 mmol) of **15b** in 45 mL of MeOH was stirred for 3 h in the presence of 0.37 g (3.49 mmol) of Na_2CO_3 in 5 mL of H_2O. After preparation as described for **4**, the product was purified by flash chromatography (silica gel; 10% ethyl acetate/petroleum ether) to give 0.10 g (34%) of **16** as a gum: ^1H NMR ($CDCl_3$) δ 0.83 (t, 3 H, ω-CH_3), 1.73 (s, 3 H, H-7), 4.10 (m, 1 H, H-3), 5.57 (s, 1 H, H-2), 5.87 (s, 1 H, H-9), 6.17 (s, 1 H, H-9), 6.13 (s, 2 H, Ar H), 9.30 (s, 1 H, H-10); IR ν_{max} (film) 3400, 1660 cm^{-1}.

10-[*N*,*N*-Bis(2-chloroethyl)amino]cannabidiol (6). A solution of 90 mg (0.27 mmol) of **16** in 20 mL of dry MeOH was added to 1 g of 3-Å molecular sieves (activated by drying at 250 °C/0.1 mm for 6 h) at room temperature. After 15 min, 250 mg (1.40 mmol) of *N,N-bis*(2-chloroethyl)amine hydrochloride was added. After an additional 15 min, 28 mg (0.44 mmol) of $NaCNBH_3$ was added and the reaction mixture was stirred for 4 h. After filtration through Celite and washing with MeOH, the filtrate was concentrated in vacuo. The residual solid was partitioned between CH_2Cl_2 and a saturated NH_4Cl solution. The organic layer was separated, dried (Na_2SO_4), and concentrated in vacuo to give a residue which was purified by flash chromatography (silica gel; 10% ethyl acetate/petroleum ether) to give 32 mg (25%) of **6** as a yellow gum: ^1H NMR ($CDCl_3$) δ 0.88 (t, 3 H, ω-CH_3), 1.80 (s, 3 H, H-7), 2.50–2.80 (m, 6 H), 3.40 (t, 4 H), 5.01 (s, 2 H, H-9), 5.60 (s, 1 H, H-2), 6.30 (s, 2 H, Ar H); IR ν_{max} (film) 3400 cm^{-1}; MS *m/z* 453, 455, 457 (M^+). Anal. ($C_{25}H_{37}Cl_2NO_2 \cdot {}^1/_4H_2O$) C, H, N, Cl.

12-Amino-Δ^8-tetrahydrocannabinol (8). To a solution of 12-hydroxy-Δ^8-THC[5b] (66 mg, 0.199 mmol) in 20 mL of CH_2Cl_2 was added 61 μL of 2,4,6-collidine while the mixture was stirred under N_2 at room temperature. After cooling of the solution to 0 °C, 70 μL of trifluoromethanesulfonic anhydride was added and the solution was stirred for an additional 0.5 h at 0 °C. The reaction mixture was diluted with 20 mL of CH_2Cl_2 and the organic layer was separated and washed successively with 20 mL each of cold 1 N HCl, H_2O, and brine. After drying, the CH_2Cl_2 was evaporated in vacuo to leave a brown gum, which was dissolved in 15 mL of DMSO and treated with 28 mg of sodium azide. After stirring overnight at room temperature, the solution was poured into 100 mL of H_2O, and the solution was extracted with ether (3 × 50 mL). After drying the ether was removed in vacuo to leave a gum, which was purified by flash chromatography (silica gel; 40% ethyl acetate/petroleum ether). The 12-azido-Δ^8-THC obtained (14 mg; 20%) was a colorless gum.

The azide obtained was reduced with $LiAlH_4$. A solution of 12-azido-Δ^8-THC (189 mg, 0.53 mmol) in 20 mL of dry ether was added dropwise to a 0 °C suspension of $LiAlH_4$ (100 mg, 2.63 mmol) in 20 mL of dry ether. The mixture was refluxed for 0.5 h. After quenching (H_2O), the mixture was filtered and the solvent was evaporated to give a residue, which was purified by flash chromatography (silica gel; 10% ethyl acetate/CH_2Cl_2) to give pure amine **8** as a light pink solid (142 mg; 81%); mp 166–167 °C; IR ν_{max} (film) 3360, 2900, 1590 cm^{-1}. Anal. ($C_{21}H_{31}NO_2$) C, H, N.

11-(*N*-Ethylamino)-Δ^8-tetrahydrocannabinol Hydrochloride (9). A solution of 11-oxo-Δ^8-THC acetate (**13**,[18] 750 mg, 2.0 mmol) under N_2 in MeOH (30 mL, dry) containing 3-Å sieves (4 g, activated at 300 °C/0.1 mm) was stirred for 15 min at room temperature. Ethylamine hydrochloride (810 mg, 10.0 mmol, 5 equiv) and $NaCNBH_3$ (150 mg, 2.4 mmol, 1.2 equiv) were added, and the mixture was stirred for 72 h. The reaction was then filtered through a pad of Celite and washed with MeOH (150 mL). The solvent was removed in vacuo and the residue was dissolved in $CHCl_3$ (200 mL), NH_4Cl (50 7L, saturated aqueous), and H_2O (200 mL). The organic layer was successively washed with NH_4Cl (25 mL), H_2O (2 × 100 mL), and NaCl (100 mL, saturated aqueous). After drying (Na_2SO_4) and evaporation in vacuo, the residue was dissolved in MeOH (250 mL) to which solid K_2CO_3 (500 mg) and H_2O (100 mL) were added, and the mixture was stirred under N_2 at room temperature for 24 h. The reaction was neutralized with NH_4Cl (5 mL, saturated aqueous) and solvent was removed in vacuo. The oil (350 mg) was taken up into a $CHCl_3$ (100 mL) and H_2O (100 mL) mixture, then the organic layer was separated, washed with H_2O (100 mL) and NaCl (50 mL, saturated aqueous), and dried (Na_2SO_4). After evaporation in vacuo the residual amine was purified by flash chromatography (silica gel; $CHCl_3$/MeOH/*n*-propylamine, 10:1:0.1; R_f = 0.25) to give **9** as the free amine (280 mg; 49%). It was converted to the hydrochloride salt ($CHCl_3$/ethereal HCl) as a purple solid: mp 100–120 °C (softening with complete melting at 120 °C); ^1H NMR ($CDCl_3$) δ 0.95 (t, 3 H), 1.0 (s, 3 H), 1.20 (s, 3 H), 1.6–3.0 (m, 10 H), 3.20 (br, s, 2 H), 6.80 (br, s, 1 H), 6.10 (s, 1 H), 6.18 (s, 1 H), 7.50 (s, 1 H), 9.0 (br, s, 2 H); IR ν_{max} (film) 1430, 1575, 1630, 2930, 3260 cm^{-1}. Anal. ($C_{23}H_{35}NO_2 \cdot HCl \cdot H_2O$) C, H, N, Cl.

11-[*N*,*N*-Bis(2-chloroethyl)amino]-Δ^8-tetrahydrocannabinol Acetate Hydrochloride (10). From a solution of **13** (1.0 g, 2.7 mmol) with *N,N*-bis(2-chloroethyl)amine hydrochloride (2.4 g, 13.6 mmol, 5 equiv) and $NaBH_3CN$ (200 mg, 3.2 mmol) amine of **10** was prepared by using the same procedure as with **9** (except without hydrolysis of the acetate group using K_2CO_3). The free amine of **10** was purified by flash chromatography (silica gel; 10% ethyl acetate/petroleum ether; R_f = 0.45) to give 0.35 g (20%) of a gum: ^1H NMR ($CDCl_3$) δ 0.80 (t, 3 H), 1.0 (s, 3 H), 1.30 (s, 3 H), 1.40–2.1 (m, 4 H), 2.20 (s, 3 H), 2.30–2.60 (m, 5 H), 2.80 (t, 4 H), 3.0 (s, 2 H), 3.40 (t, 4 H), 5.50 (br, s, 1 H), 6.3 (d, 1 H), 6.45 (d, 1 H); IR ν_{max} ($CDCl_3$) 1215, 1425, 1575, 1780, 2940 cm^{-1}. The hydrochloride salt was prepared in the same manner as with **9**; mp 60–70 °C. Anal. ($C_{27}H_{39}NO_3Cl_2 \cdot HCl$) C, H, N, Cl.

12-(*N*-Ethylamino)-Δ^8-tetrahydrocannabinol (11). A solution of 232 mg (0.70 mmol) of **8** and 107 mg (0.35 mmol) of tetraacetylglycoluril[19] in 60 mL of CH_3CN was heated at 50 °C for 50 h under N_2. The reaction mixture was cooled to room temperature, poured into 100 mL of H_2O, and extracted with ether (4 × 80 mL). The combined ether extracts were washed with brine (2 × 50 mL), dried (Na_2SO_4), and concentrated in vacuo to give a residue which was purified by flash chromatography (silica gel; 10% ether/CH_2Cl_2) to provide 155 mg (59%) of 12-acetamido-Δ^8-THC as a yellow foam: mp 88–90 °C; MS *m/z* 371 (M^+), 299, 231; IR ν_{max} (film) 3300, 1620 cm^{-1}.

A solution of this amide (155 mg, 0.41 mmol) in 10 mL of dry THF was added to a stirred suspension of 100 mg (2.6 mmol) of $LiAlH_4$ in 20 mL of dry THF at 0 °C under N_2. After refluxing the mixture for 20 h, the reaction was quenched by the addition of H_2O at 0 °C. The mixture was filtered and the precipitate was washed with ether. After evaporation of the solvent in vacuo, the residue was purified by flash chromatography (silica gel; 5% MeOH/CH_2Cl_2) to provide 112 mg (75%) of **11** as a colorless oil; IR ν_{max} (film) 3300, 2900, 1560, 1440 cm^{-1}. Anal. ($C_{23}H_{35}NO_2 \cdot {}^1/_4H_2O$) C, H, N.

11-Oxo-Δ^8-tetrahydrocannabinol (14). To a solution of 11-oxo-Δ^8-THC acetate (**13**)[18] (1.0 g, 2.7 mmol) in 100 mL of MeOH was added 10 mL of 5% K_2CO_3 solution, and the mixture was stirred for 2 h at room temperature under N_2. The reaction was quenched by the addition of 25 mL of NH_4Cl (saturated aqueous) with stirring for 15 min. The solvent was removed in vacuo and the residue was dissolved in 100 mL of CH_2Cl_2, which was washed successively with 100 mL each of aqueous concetrated NH_4Cl, H_2O, and aqueous, concetrated NaCl and then dried and evaporated in vacuo to give **14** (0.8 g; 91%): ^1H NMR ($CDCl_3$) δ 0.90 (br, t, 3 H), 1.05 (s, 3 H), 1.20 (s, 3 H), 2.0–3.0 (m, 10 H), 3.95 (br, d, 1 H), 6.03 (s, 1 H), 6.10 (s, 1 H), 6.80 (s, 1 H), 9.50 (s, 1 H). It was used without purification in the subsequent reaction.

11-[*N*,*N*-Bis(2-chloroethyl)amino]-Δ^8-tetrahydrocannabinol (12). To a solution of **14** (0.9 g, 2.7 mmol) in 200 mL of dry MeOH containing 3-Å molecular sieves (9 g; activated at 300 °C/0.1 mm) and stirred for 15 min at room temperature under N_2 were added *N*,*N*-bis(2-chloroethyl)amine hydrochloride (2.5 g, 13.8 mmol, 5 equiv) and $NaCNBH_3$ (0.28 g, 4.4 mmol, 1.6 equiv). This mixture was stirred for 14 h. After filtration through Celite and washing with 200 mL of MeOH, the solvent was removed in vacuo and the residue was dissolved in a mixture of 300 mL of CH_2Cl_2 and 100 mL of NH_4Cl (saturated aqueous). The organic layer was washed with brine, dried, and evaporated in vacuo. The residue was purified by flash chromatography (silica gel; 20% ethyl acetate/petroleum ether) to give 0.33 g (27%) of **12**: ^1H NMR ($CDCl_3$) δ 0.90 (t, 3 H), 1.05 (s, 3 H), 1.15 (s, 3 H), 1.60–2.60 (m, 10 H), 2.85 (t, 4 H), 3.05 (s, 2 H), 2.50 (t, 4 H), 4.90 (s, 1 H), 5.65 (s, 1 H), 6.07 (d, 1 H), 6.15 (d, 1 H); IR ν_{max} ($CDCl_3$) 1430, 1580, 1625, 2935, 3610 cm^{-1}. Anal. ($C_{25}H_{37}Cl_2NO_2$) C, H, N, Cl.

Pharmacology. Materials. Male ICR mice (22–30 g), obtained from Dominion Laboratories (Dublin, VA), were maintained on a 14/10-h light/dark cycle and received food and water ad libitum. Cannabidiol, Δ^8-THC, and Δ^9-THC were obtained from the National Institute on Drug Abuse.

Drug Preparation and Administration. The procedure of Olson et al.[10] was used to prepare 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), or intraventricularly in a volume of 5 μL by using previously described methods.[11]

Behavioral Evaluations. Spontaneous locomotor activity, antinociception (via tail-flick latency), hypothermia, and catalepsy (via the ring-test) were evaluated by previously reported methods.[6,12,13] Besides vehicle control, Δ^9-THC was administered as a positive control in each daily experiment, and a dose–response curve for Δ^9-THC was generated.

Possible antagonistic properties of the cannabinoids were also determined by previously reported methods.[6,12,13] Mice were pretreated with **2**, **3**, **4**, **5**, **8**, **9**, **10**, or **12** (at doses of 30, 1, 3, 1, 3, 3, 50, and 50 mg/kg intravenously, respectively) or 30 mg/kg 10-bromo-CBD diacetate or with **6** or **12** (at a dose of 150 μg intraventricularly) 10 min prior to administration of 6 mg/kg Δ^9-THC. Additionally, antagonism by **10** and **12** was also evaluated by administering Δ^9-THC at 24 and 48 h after nitrogen mustard treatment.

Statistical analysis was performed by using ANOVA (with Dunnett's *t* test for comparisons to control, and Scheffe's *F* test for multiple comparisons), and differences were considered significant at the $p < 0.05$ level (two-tailed).

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Registry No. **2**, 22972-55-0; **3**, 92974-69-1; **4**, 112812-70-1; **4**·HCl, 125590-92-3; **4a**, 125519-11-1; **5**, 112812-71-2; **6**, 112824-01-8; **7** (R = OH), 92464-59-0; **8**, 125519-07-5; **8** (R = N_3), 125519-14-4; **9**, 112812-74-5; **9**·HCl, 125519-12-2; **10**, 112812-75-6; **10**·HCl, 125519-13-3; **11**, 125519-08-6; **11** (R = HNAc), 125519-15-5; **12**, 112812-76-7; **13**, 51263-83-3; **14**, 53865-18-2; **15a**, 125519-09-7; **15b**, 57361-62-3; **15** (R = CH_2Br), 81780-81-6; **15** (R = CH_2N_3), 95647-69-1; **16**, 125519-10-0; $HN(C_2H_4Cl)_2$·HCl, 821-84-7; $H_2NC_2H_5$, 75-04-7; $H_2NC_3H_7$, 107-10-8; tetraacetylglycoluril, 10543-60-9.

Synthesis, Conformation, and Immunosuppressive Activity of Cyclosporines That Contain ε-Oxygen (4*R*)-4-[(*E*)-Butenyl]-4,*N*-dimethyl-L-threonine Analogues in the 1-Position[1]

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A series of CsA analogues that contain novel ε-oxygen isosteres of (4*R*)-4-[(E)-butenyl]-4,*N*-dimethyl-L-threonine (MeBmt) in the 1-position were synthesized. The key steps for the syntheses of enantiomerically pure ε-oxygen MeBmt analogues **4**–**7** were based on the stereoselective epoxidation of *cis*-allylic alcohol derivative **12** with a peracid, followed by the application of a base-catalyzed intramolecular rearrangement of epoxyurethane **15**, which was derived from the reaction of epoxy alcohol **14** and methyl isocyanate. All ε-oxygen MeBmt analogues have the same stereochemistry and the same functional groups as those on the α,β,γ-carbons of MeBmt except for the double bond of MeBmt, which is replaced by the $-OCH_2-$ group. The syntheses of the peptide portion of CsA analogues followed the strategy we reported previously.[10b,16] The immunosuppressive activities of CsA analogues **28a**–**e**, determined by inhibition of concanavalin A stimulated thymocytes, showed that **28b**, which has the closest structural resemblance to MeBmt, retains about 7–10% of activity of CsA, whereas the analogues **28a**, **28c**, and **28e** retain about 2–5% activity. It is interesting to note that **28d**, which has the larger benzyl group on the end of the side chain, is about 20–25% as active as CsA. Extensive conformational analyses by 1D and 2D NMR indicated that the conformation of the 33-membered peptide ring system for all CsA analogues was very similar to that of CsA. However, the NMR analyses revealed that the 1-position side chain of all these CsA analogues adopted a novel conformation in chloroform by forming a different intramolecular hydrogen bond between the β-OH and the ε-oxygen of the same residue. The NMR data also suggest that the chloroform conformation of these CsA analogues is similar to the conformation observed in the crystal structure of CsA in that the 1-position side chain is folded across the cyclic peptide ring system.

Since its introduction on the market only a few years ago, cyclosporine A (**1**; Sandimmune)[2] has become an important drug for preventing rejection of transplanted human organs.[3] In addition to its well-established immu-

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