Some 9-Hydroxycannabinoid-like Compounds. Synthesis and Evaluation of Analgesic and Behavioral Properties

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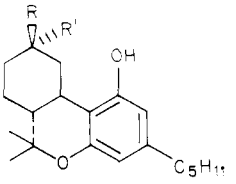
A series of 9-hydroxylated cannabinoid-like compounds was prepared and tested for analgesic properties in mice and behavioral properties in dogs. Although the prototype compound, 9-nor-9-hydroxyhexahydrocannabinol, has potent antinociceptive activity in laboratory animals, the new analogues were relatively inactive. All of the compounds produced an alteration of behavior in unanesthetized dogs. Two of the compounds produced cannabinoid-like effects and the other two produced general CNS depression.

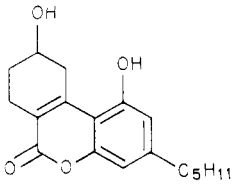
We have previously reported that (−)-9-nor-9β-hydroxyhexahydrocannabinol (**1a**) has analgesic potency



1a, R = OH; R' = H
b, R = H; R' = OH

in mice similar to morphine in the hot-plate test and is slightly less potent than morphine in the Nilsen test.[3] Subsequently, **1a** has been found to be about 5 and 20 times as potent as morphine in mice in the tail-flick and *p*-phenylquinone-writhing tests, respectively.[4] Interestingly, the epimer of **1a**, 9-nor-9α-hydroxyhexahydrocannabinol (**1b**), is inactive in all of the above analgesic tests at much higher doses. However, **1a** and **1b** produce behavioral effects in dogs and mice with **1a** only slightly more potent than **1b**.[4]

Inasmuch as the 9β-hydroxy group appears necessary for the analgesic activity, some additional synthetic compounds which maintain or approximate this configuration of the 9-hydroy group have been prepared and examined for analgesic and behavioral properties. Those compounds prepared and tested are **2**–**5**. Compound **2**



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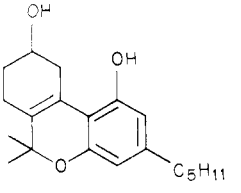

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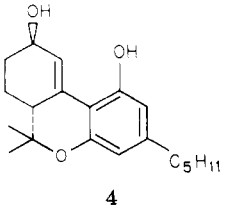

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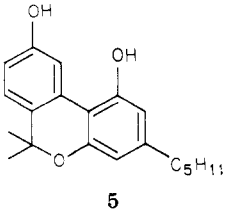

5

(a $\Delta^{6a,10a}$-THC) was of particular interest because it has been reported that a carbonyl at the 6 position instead of a *gem*-dimethyl in $\Delta^{6a,10a}$-THC's caused a loss of CNS behavioral effects.[5] Since our results with **1a** and **1b** have suggested that behavioral effects and analgesic effects of these compounds may result from interaction with different receptors or by different mechanisms, it was of interest to see if **2** would be devoid of behavioral side effects while retaining the analgesic effect. Synthetically, **2** and **3** have shorter routes than **1a** and avoid the separation of epimeric alcohols and mixtures of cis- and trans-fused ring isomers. Compound **4** also avoids the separation of cis- and trans-fused ring isomers but must be prepared free of its epimeric alcohol. Compound **5**, in which the cyclohexane ring of **1a** has been aromatized, is actually a structural derivative of the weakly analgesic compound, cannabinol (CBN).[6]

Chemistry. Compound **2** was prepared in good yield by $NaBH_4$ reduction of known ketone **6**.[7] Treatment of **2** with methylmagnesium chloride gave the intermediate alcohol **7**, which was not isolated but cyclized directly to **3** with acid. In the 100-MHz NMR of **2**, H_9 was coupled by 7 Hz to one H_{10} proton and 4 Hz to the other H_{10} proton. Similarly, for compound **3**, H_9 is coupled by 5 and 8 Hz to the two H_{10} protons. Unfortunately, this does not allow an unequivocal assignment of the conformation of the 9-hydroxyl in these compounds. However, examination of molecular models suggests that a nearly equatorial conformation should be energetically favorable and this is consistent with the NMR data.

Compound **4** was prepared by the $NaBH_4$ reduction of known ketone **8**.[7] In the preparation of **1a** from the corresponding ketone using $NaBH_4$, over 90% of the product was **1a**.[4] Similarly, the reduction of **8** gave a major and a minor component. If the stereochemistry of the reduction of **8** is the same as in the preparation of **1a**, then compound **4** should be the major product. In the 100-MHz NMR spectrum of the major product, the peak half-width of H_9 was 16 Hz, suggesting the presence of diaxial coupling to one of the adjacent protons. Therefore, it would appear that the 9-hydroxy of this material is more nearly equatorial than axial and the structure is that shown for **4**.

Compound **5** was also prepared starting from ketone **8**. A recently reported procedure for aromatizing α-alkylcyclohexanones was applied to enone **8**.[8] Heating **8** in acetic acid–acetic anhydride with 1 mol of sulfuric acid gave the desired aromatized product as the diacetate **9**. Base hydrolysis then gave **5** in good overall yield from **8** (see Scheme I). The 9-hydroxy in **5** lies in the plane of the aromatic ring and, thus, would more closely approximate the equatorial 9-hydroxy of **1a** than the axial 9-hydroxy of **1b**. In **5**, the 9-hydroxy is also phenolic and should be more acidic than the 9-hydroxy of **1a**.

Pharmacological Methods. Cannabinoids produce a very characteristic effect on the overt behavior of dogs,

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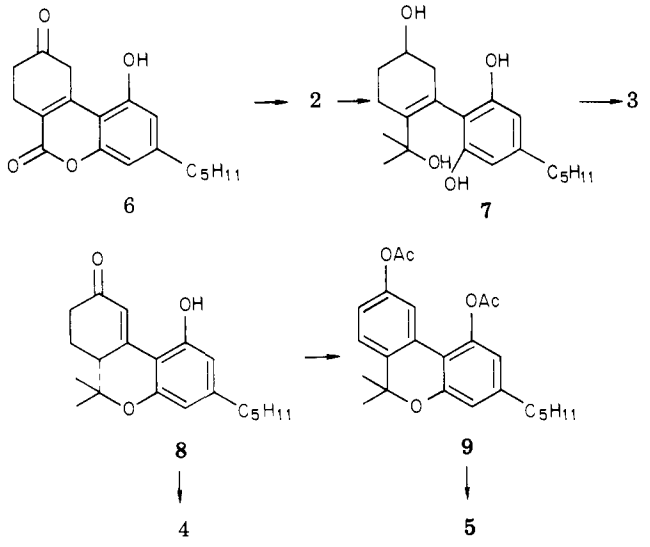
Table I. Effect of Δ^9-THC and Synthetic Compounds on Overt Behavior in Dogs[a]

dose, mg/kg	Δ^9-THC	(-)-1a	(+)-1a	(+)-1b	2	3	4	5
0.05		1+ (3)[b]						
0.10	1+ (3)	3 (3)	3 (2)					
0.20	3+ (2)					1 (2)		
0.40	4 (2)							
0.50		5+ (2)		3- (2)	1- (4)[c]	2 (3)		
1.00						5- (2)	1 (2)	
2.00					6 (3)[d]		5- (2)	2- (4)[c]
4.00							6 (2)	
10.00								6 (2)[d]

[a] Semiquantitated by the dog ataxia rating scale (see Pharmacological Methods and ref 9). [b] The mean score of all animals tested is presented with the number of animals tested in parentheses. [c] The activity was depressed. There were few cannabinoid signs. [d] These dogs became prostrate. The effects appeared to be sedation and muscle relaxation, but there were no cannabinoid effects in the classical sense.

Scheme I



including static ataxia which can best be described as swaying forward and backward and from side to side without movement of the feet.[9] Other effects of cannabinoids in dogs include hyperreflexia; decreased spontaneous activity; and, at higher doses, prostration. A more detailed description of the behavioral effects of cannabinoids in dogs has been presented.[10,11] The severity of the response is dose related. The effects of the compounds reported in this paper were semiquantitated in dogs using the methodology and behavioral rating scale reported elsewhere.[9] Briefly, three independent observers rated the effect of each dose of the drug on each dog after IV administration using a scale of zero (no effect) to six (dog lies prostrate on floor), and the mean of their scores were recorded. The score at the time of peak activity is reported here.

All compounds were examined by sc administration for analgesic activity in the mouse hot-plate test[12] which we have previously found useful for screening a large number of cannabinoid compounds.[13] Compound **5** was also examined sc in the mouse tail-flick test[14] for analgesia.

All compounds were given at a volume of 0.1 mL/kg to dogs and 0.1 mL/g to mice as a suspension in Emulphor (El-620), ethanol, and saline.[15]

Results

The effects of these compounds on the overt behavior of dogs are shown in Table I. For comparison **1a**, **1b**, and Δ^9-tetrahydrocannabinol (Δ^9-THC) are also included. All compounds produced prostration at the higher doses tested, as shown in Table I. Of the new compounds reported in this paper, compound **3** is the most potent. It was essentially equipotent with **1b**. Compound **4** was about one-half as active as **3**. Both **3** and **4** gave the typical cannabinoid-like syndrome, including hyperreflexia and static ataxia.

Table II. Analgesic Data

compd	analgesic ED_{50}, mg/kg[a] hot plate	tail flick
(-)-1a	1.6 (1.3-1.8)	1.0 (0.61-1.65)
(±)-1a	2.9 (2.3-3.6)	
2	NA[b]	
3	NA[c]	
4	NA[c]	
5	35.6 (21.8-58.0)	NA[d]
morphine hydrochloride	1.2 (0.9-1.3)	5.8 (5.68-5.92)

[a] Ninety-five percent confidence limits are shown in parentheses. [b] Not active up to 20 mg/kg. [c] Not active up to 50 mg/kg. [d] Not active up to 10 mg/kg.

Compounds **2** and **5** both produced depression which differed significantly in its overall profile from that produced by compounds **3** and **4**. Neither **2** nor **5** produced the cannabinoid-like syndrome of the static ataxia and other effects described previously. The effects of these two compounds more nearly resembled those seen after sedative–hypnotic drugs, such as barbiturates, which induce increasing degrees of sedation and muscular relaxation leading to prostration at higher doses.

The results of analgesic testing are shown in Table II. The only compound possessing analgesic activity in the hot-plate test was **5**. However, this activity was very weak compared to the previously tested compounds shown in Table II. In the tail-flick test, compound **5** was inactive at 10 mg/kg.

Discussion

All of the compounds reported in this paper produced behavioral effects in dogs. Compounds **3** and **4** produced classical cannabinoid-like syndromes, with compound **3** approximately equipotent with **1b** and slightly more potent than **4**. Perhaps the most significant result of testing these compounds in dogs is the activity of compound **2**. It was previously reported that the analogue of **2** in which the 9-hydroxy is replaced by a 9-methyl was devoid of CNS effects.[4] However, we have found that the hydroxy compound **2** has sedative–hypnotic properties in dogs. Also, this compound appears to lack many of the behavioral side effects seen with the cannabinoids in this test. This compound would, therefore, appear to represent a separation of the cannabinoid CNS depressant effects from many of the other cannabinoid behavioral effects. If compound **2**, like the cannabinoids, has little or no physical

dependence liability, it may represent a new class of CNS depressants.

Similar to compound **2**, compound **5** produced a noncannabinoid-like depression in dogs. Although compound **5** was found to be less potent than the other compounds, the observed depressant activity is significant because cannabinol (which differs from **5** by substitution of a methyl for the 9-hydroxy) is reported to be inactive in dogs and several other species.[16] Therefore, we have found that substitution of a hydroxyl for a methyl, as in **2** and **5**, has given compounds with interesting biological activity as compared with their inactive precursors.

Interestingly, none of the compounds tested had analgesic properties similar to **1a**. The inactivity of **1b** and the four new compounds presented here indicates that the structural requirements for analgesia in these compounds are very strict. In contrast, opiate analgesia can accommodate a wide variety of structural variations.[17] Inasmuch as the compounds reported here all have activity in the dog ataxia test, they presumably reach the brain, including the sites where **1a** produces analgesia. Therefore, it must be concluded that these compounds fail to meet the structural requirements for analgesia, but we have not ruled out the possibility that they might bind the "analgesic receptors" in an antagonist manner.[18]

Experimental Section

The assigned structures of all compounds were supported by their NMR, IR, and mass spectra. Spectral and elemental analyses were performed by the Section on Analytical Services and Instrumentation (NIAMDD, NIH). NMR spectra were recorded on a Varian HA-100. Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values. All melting points were taken on a Thomas-Hoover melting point apparatus and are uncorrected.

(±)-6,6,9-Trinor-6-oxo-$\Delta^{6a,10a}$-tetrahydrocannabinol (2). To 1.0 g (0.0033 mol) of ketone **6** slurried in 50 mL of MeOH under a stream of N_2 was added 0.125 g (0.003 mol) of $NaBH_4$ and the mixture was stirred for 15 min. The yellow mixture was then made slightly acidic by the dropwise addition of 10% HCl and then evaporated to a gummy mass. The residue was dissolved in ether which was washed well with H_2O and NaCl–H_2O, dried ($MgSO_4$), and evaporated to give 1.0 g of white solid. Recrystallization from Me_2CO gave 0.688 g (69%) of white crystals: mp 181–183 °C; NMR (CD_3OD) δ 4.05 (br, H_9), 3.60 (q, $J_{9,10\alpha}$ = 4.0 Hz, $J_{10\alpha,10\beta}$ = 20.0 Hz, $H_{10\alpha}$), 3.10 (q, $J_{9,10\beta}$ = 7.0 Hz, $J_{10\alpha,10\beta}$ = 20.0 Hz, $H_{10\beta}$). Anal. ($C_{18}H_{22}O_4$) C, H.

(±)-9-Nor-9-hydroxy-$\Delta^{6a,10a}$-tetrahydrocannabinol (3). To 3.0 g (0.01 mol) of lactone **2** in 50 mL of dry THF was added 33 mL of 3 M MeMgCl in THF (Fisher) and the mixture was refluxed 2 days under N_2. After cooling the mixture, 10 mL of 10% HCl was carefully added followed by 15 mL of 6 N HCl, and the mixture was stirred for 3 h at room temperature and then for 30 min at 40–45 °C. This mixture was extracted with ether, which was then washed with H_2O, dried ($MgSO_4$), and evaporated to a green gum. Crystallization from ether–ligroin (bp 30–60 °C) gave, initially, 1.5 g of greenish crystals followed by 0.3 g of white crystals. Recrystallization of the latter gave the analytical sample, mp 134–135 °C. Chromatography of the greenish crystals over silica gel using ligroin gave an additional 1.0 g as light tan crystals: mp 134–135 °C; NMR (CD_3OD) δ 3.81 (br, H_9), 2.97 (q, $J_{9,10\alpha}$ = 5.0 Hz, $J_{10\alpha,10\beta}$ = 18.0 Hz, $H_{10\alpha}$), 2.64 (q, $J_{9,10\beta}$ = 8.0 Hz, $J_{10\alpha,10\beta}$ = 18.0 Hz, $H_{10\beta}$). Anal. ($C_{20}H_{28}O_3$) C, H.

(±)-9-Nor-9β-hydroxy-Δ^{10}-tetrahydrocannabinol (4). To 0.5 g (0.0016 mol) of ketone **8** slurried in 25 mL of MeOH under a stream of N_2 was carefully added 0.15 g (0.004 mol) of $NaBH_4$, and the mixture was stirred for 3 h. Then, 50 mL of ether was added and the mixture was made slightly acidic with 5% H_2SO_4 while being cooled. The ether layer was washed with H_2O, NaCl–H_2O, and dried ($MgSO_4$). Then the ether was concentrated under a stream of N_2 (heating caused extensive decomposition) until crystals just began to form, and then ligroin (bp 30–60 °C) was added and the mixture cooled. This gave 0.238 g (48%) of white crystals: mp 141–143 °C (dec); NMR (CD_3OD) δ 7.06 (s, br, olefinic, H_{10}), 4.33 (br, peak half-width = 16 Hz, H_9). Anal. ($C_{20}H_{28}O_3$) C, H.

9-Nor-9-acetoxycannabinol Acetate (9). To 0.3 g (0.001 mol) of a cooled solution of ketone **8** in 8 mL of AcOH and 8 mL of Ac_2O was added dropwise 0.09 g (0.001 mol) of H_2SO_4 in 0.5 mL of AcOH. The mixture was then refluxed for 1 h and stirred overnight at room temperature under N_2. After stirring for 0.5 h with 25 mL of ice and H_2O, the mixture was extracted with 4 × 50 mL of Et_2O. The Et_2O was washed well with 5% $NaHCO_3$, H_2O, and NaCl–H_2O, dried ($MgSO_4$), and evaporated to give a viscous dark-orange oil. Chromatography of the residue over silica gel using $CHCl_3$ gave 0.23 g (61%) of a colorless oil which crystallized on standing. Recrystallization from hexane gave colorless plates: mp 67–69 °C; IR (CCl_4) 1775 cm^{-1}; NMR (CCl_4) δ 2.26 (s, 3 H, acetate), 2.22 (s, 3 H, acetate). Anal. ($C_{24}H_{28}O_5$) C, H.

9-Nor-9-hydroxycannabinol (5). The crude diacetate **9** obtained from 2.0 g (0.006 mol) of ketone **8** using the above procedure was dissolved in 50 mL of MeOH and cooled, and 10 mL of 10% NaOH was added. The mixture was stirred for 30 min under N_2 at room temperature and then 15 mL of 10% HCl was added. This was extracted with Et_2O–ligroin (bp 30–60 °C) to give 0.786 g (40% based on ketone **8**) of light pink crystals: mp 188–190 °C; NMR (CD_3OD) δ 8.08 (d, H_{10}, $J_{8,10}$ = 3 Hz), 7.07 (d, H_7, $J_{7,8}$ = 8 Hz), 6.65 (q, H_8, $J_{7,8}$ = 8.0 Hz, $J_{8,10}$ = 3 Hz), 6.34 (d, H_2 or H_4, J = 1.5 Hz), 6.28 (d, H_2 or H_4, J = 1.5 Hz). Anal. ($C_{20}H_{24}O_3$) C, H.

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References and Notes

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