Notes

Synthesis and Pharmacological Properties of 11-Hydroxy-3-(1′,1′-dimethylheptyl)hexahydrocannabinol: A High-Affinity Cannabinoid Agonist

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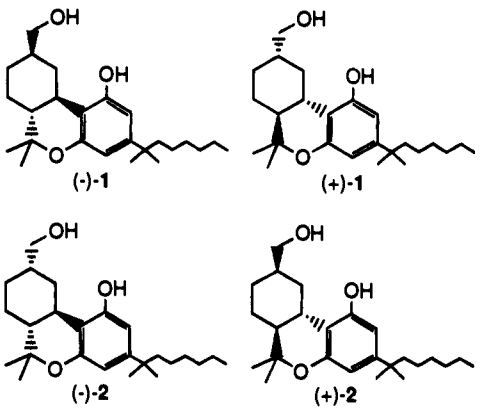
11-Hydroxy-3-(1′,1′-dimethylheptyl)hexahydrocannabinol (**1**) was synthesized from the known cannabimimetic analog (±)-nabilone. Racemic **1** was resolved by HPLC on a semipreparative CHIRALCEL OD column (Daicel, Inc.), and pharmacological activities of the individual enantiomers were evaluated in the mouse model. The (−)-enantiomer was found to be much more potent than the (+)-enantiomer in all the four measures with the potency ratios in the production of catalepsy (RI), hypoactivity (SA), hypothermia (RT), and antinociception (TF) being 93, 143, 186, and 322, respectively. The racemic 11α-OH diastereomer (**2**), a reaction side product, was also evaluated in the mouse model. Only small differences in the pharmacological activity of racemic **1** and **2** were found in the above four measures.

The search for novel high-affinity cannabimimetic ligands has led to the synthesis of numerous cannabinoid analogs and the establishment of structure–activity relationships for cannabinoid activity.[1] Recently, 11-hydroxy-3-(1′,1′-dimethylheptyl)hexahydrocannabinol (1) was reported as a novel probe for the cannabinoid receptor.[2] It was synthesized by hydrogenation of 11-hydroxy-3-(1′,1′-dimethylheptyl)-Δ^8-THC and was found to possess the highest affinity for the cannabinoid receptor of all analogs reported to date. Indeed, the receptor binding experiments[2] have shown that it binds strongly to the cannabinoid receptor with a K_D of 45 pM, which is considerably lower than the K_i of 2.0 nM reported for CP-55940, the ligand typically used for studying the cannabinoid receptor. The present paper describes a different synthetic approach to 1 which has some distinct advantages over the method mentioned above. As the starting material for this synthetic route, we have used racemic nabilone, a cannabimimetic agent synthesized by Eli Lilly and Co.[3] The racemic mixture of the final compound was then separated into individual enantiomers using chiral HPLC. Furthermore, the 11α-OH diastereomer **2** was a reaction side product and could thus be isolated. This has allowed us to subject the enantiomeric pair as well as the two racemic diastereomers to the four pharmacological tests now used routinely to characterize cannabimimetic activity. Thus, we are now able to discuss in some detail the pharmacologic enantioselectivity of 1 and also to compare it with its less active axial diastereomer **2** in a mouse model for cannabimimetic activity.



Chemistry

The key step in the synthesis of 11-hydroxy-3-(1′,1′-dimethylheptyl)hexahydrocannabinol (1) from readily available (±)-nabilone (**3**) (Scheme 1) was the *Wittig–Horner–Emmons* olefination of *O*-benzyl-protected nabilone (**4**) with diethyl (α-lithioisocyanomethyl)phosphonate prepared from diethyl (isocyanomethyl)phosphonate and *n*-butyllithium.[4] The intermediate α,β-unsaturated isocyanide (**5**) was converted, without isolation, into aldehyde **7** by acidic hydrolysis. The ratio of equatorial (**7a**) and axial (**7b**) aldehydes, obtained from the proton NMR spectrum of the product mixture, was about 3:1, respectively. We have also found that the axial aldehyde **7b** could be quantitatively epimerized to the equatorial isomer **7a** using sodium bicarbonate in methanol.[5] After separation of the two racemic epimers by column chromatography on silica gel, the benzylic protecting group was removed by hydrogenolysis over palladium on active carbon catalyst followed by reduction of the formyl group using lithium tri-*tert*-butoxyaluminohydride to afford pure (±)−1. Attempts to debenzylate the axial epimer (**7b**) under identical

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Scheme 1

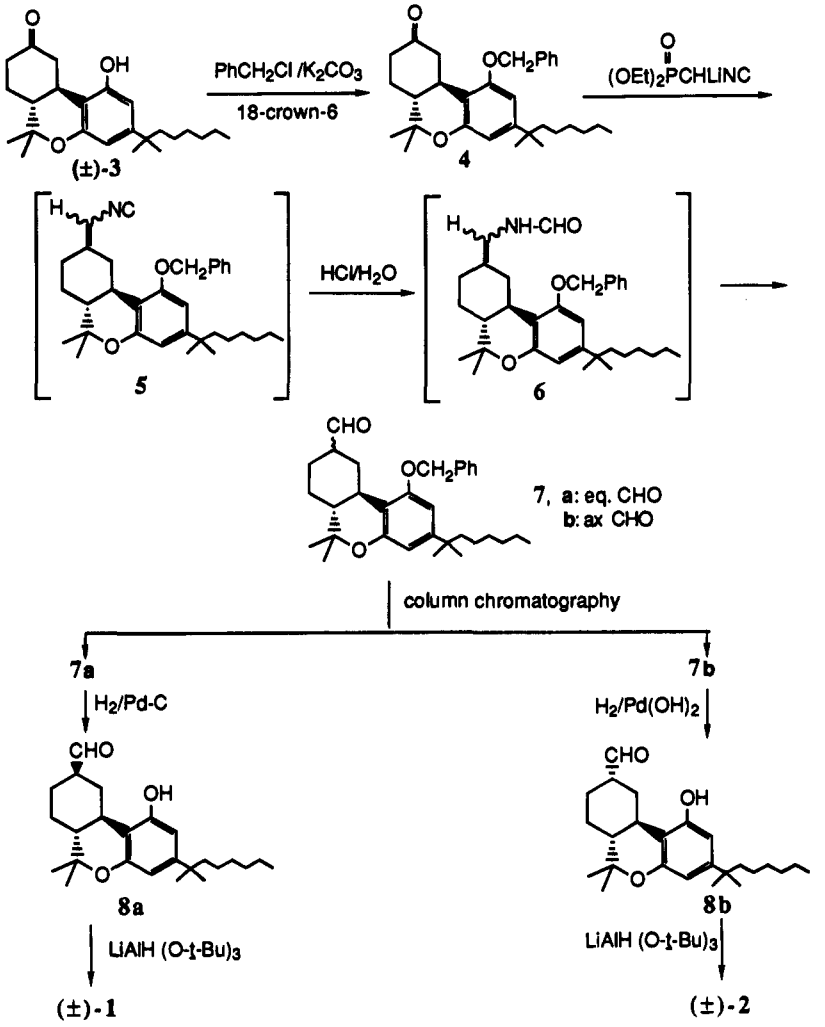

Table 1. Biological Testing of 11-Hydroxy-3-(1′,1′-dimethylheptyl)hexahydrocannabinols[a]

compd	ED50 (μg/kg) SA	TF	RT	RI
Δ^9-THC	1006 ($n = 5, R = 0.93$)	1415 ($n = 7, R = 0.97$)	1415 ($n = 7, R = 0.95$)	1510 ($n = 7, R = 0.97$)
(±)-1	10 ($n = 4, R = 0.96$)	3.2 ($n = 4, R = 0.98$)	52 ($n = 4, R = 0.97$)	69 ($n = 4, R = 0.99$)
(−)-1	5.4 ($n = 7, R = 0.97$)	1.8 ($n = 5, R = 0.97$)	37 ($n = 7, R = 0.95$)	27 ($n = 6, R = 0.96$)
(+)-1	770 ($n = 5, R = 0.93$)	580 ($n = 6, R = 0.96$)	6900 ($n = 5, R = 0.93$)	2500 ($n = 7, R = 0.96$)
(±)-2	36 ($n = 5, R = 0.93$)	20 ($n = 6, R = 0.99$)	120 ($n = 4, R = 0.99$)	84 ($n = 4, R = 0.98$)

[a] Following intravenous injection, analogs were evaluated in a mouse model for cannabinoid activity, a tetrad of evaluations measuring inhibition of spontaneous activity (SA) at 5–15 min, inhibition of tail-flick response (TF) at 20 min, reduction of rectal temperature (RT) at 60 min, and production of ring immobility (RI) at 90 min postinjection. The ED_{50} values for each evaluation were determined by log dose–response regression analysis as described elsewhere,[6] using ALLFIT to determine estimated standard error. Linear regressions of above data determined from $n = 4–7$ active doses, and the correlation coefficients for these regressions varied from $R = 0.93$ to 0.99.

hydrogenolysis conditions were unsuccessful. However, when palladium hydroxide on carbon was used as a catalyst, debenzylation occurred smoothly to afford **8b** which was subsequently reduced by lithium tri-*tert*-butoxyaluminohydride to afford pure (±)-**2**. Racemic 1 was then resolved by chiral HPLC using a CHIRALCEL OD (cellulose (3,5-dimethylphenyl)carbamate, Daicel Inc.) column to give pure (+)-1, and (−)-1. The overall isolated yield of 11β-hydroxy-3-(1′,1′-dimethylheptyl)-hexahydrocannabinol (1) using nabilone as a starting material was about 21%. However, if the epimerization of the 11α-OH isomer into its more active 11β-OH isomer is taken into account, then the overall yield is over 25%.

Biological Evaluation and Conclusions

The pharmacological activities of 1 and **2** were evaluated in the mouse (Table 1). Both compounds possess potencies much greater than that reported for the prototypic cannabinoid Δ^9-tetrahydrocannabinol (Δ^9-THC), which typically possesses ED_{50} values of 1–3 mg/kg in these four measures.[6] The most potent compound was (−)-1, with ED_{50} values between 1.8 and 37 μg/kg. This 100–1000 times shift in potency when compared to Δ^9-THC is generally consistent with the previously reported affinity of (−)-1 for the cannabinoid receptor, which was 1000 times higher than that of Δ^9-THC.[2] The corresponding enantiomer (+)-1 was shown to be much less potent, with the racemic mixture

producing the intermediate values between the two isomers, as would generally be expected. However, the differences in potency between the two enantiomers varied depending on the measure being evaluated, with potency ratios in the production of catalepsy (RI), hypoactivity (SA), and hypothermia (RT) being 93, 143, and 186, respectively. Yet the potency ratio between enantiomers in the antinociceptive measure (TF) was 322, over twice that of the average for the other three measures combined (mean ± SE; 141 ± 27). This difference is encouraging as it suggests that separation of these behavioral measures is possible.' Unfortunately, this separation is still not large enough to qualify this novel cannabimimetic as a useful analgesic agent.

Though no direct comparison of the enantiomers is possible, some comments can be made concerning the potencies of the diastereomers 1 and **2**, based upon the relative activities of the racemic mixtures. The analog possessing the axial 9-hydroxymethyl group (**2**) was nearly equipotent with its equatorial epimer in the catalepsy (RI) evaluation, approximately 6 times less potent in the antinociceptive (TF) measure, and intermediate in the remaining measures. These small differences in pharmacological activity between the axial and the equatorial isomers described here contrast earlier work with 9-nor-9α-hydroxy- and 9-nor-9β-hydroxyhexahydrocannabinol analogs. The data reported in that study indicate that although psychopharmacological activity resided in both epimers, only the 9β-hydroxy analog possessed analgesic activity.[7] Our results are more congruent with another reported study in which 9α-hydroxy- and 9β-hydroxyhexahydrocannabinol showed no difference in analgesic activity. This latter pair of diastereomers corresponds to that described in our report except that the dimethylheptyl side chain of 1 and **2** is substituted by an *n*-pentyl and consequently possess weaker pharmacological potencies (ED_{50} ~ 25 mg/kg).[8] Thus data presented here demonstrate more clearly that pharmacological selectivity is not likely to be achieved through modification of the relative stereochemistry of substituents at the C9-position.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Proton and carbon-13 nuclear magnetic resonance spectra were recorded on a Bruker WP-200sy (200-MHz) instrument and are reported in ppm from internal standard Me_4Si. High-resolution mass spectra were recorded on a KRATOS MS-902 instrument at 70 eV. Racemic nabilone was obtained from Eli Lilly and Co. Analytical thin-layer chromatography was performed on E. Merck DC-Plastikfolien Kieselegel 60 F. E. Merck silica gel 60 (230–400 mesh) was used for column chromatography. High-pressure liquid chromatography was performed on Waters Model 590 instrument equipped with a semipreparative Chiralcel OD (250 mm × 20 mm) column purchased from J. T. Baker, Inc. Optical rotations were determined on a Perkin-Elmer 241 polarimeter in chloroform.

Nabilone Benzylate (4). A heterogeneous mixture of nabilone (800 mg, 2.15 mmol), benzyl chloride (772 mg, 5.63 mmol), potassium carbonate (720 mg, 5.22 mmol), and 18-crown-6 (88 mg) in 40 mL of freshly distilled acetone was refluxed with stirring for 5 h. At the end of the time period, acetone was evaporated and the residue was dissolved in diethyl ether. The ether solution was washed with water, dried (Na_2SO_4), and rotary evaporated. The crude product was chromatographed on silica gel (40% diethyl ether–petroleum ether as eluent) to afford 924 mg (yield 93%) of the title compound: ^1H NMR ($CDCl_3$) δ 7.4 (m, 5H), 6.43 (s, 2H), 5.09 (s, 2H), 3.85 (d, 1H), 1.47 (s, 3H), 1.19 (s, 6H), 1.10 (s, 3H), 0.85 (t, 3H); HRMS calcd for $C_{31}H_{42}O_3$ 462.3134, found 426.3130.

Compound 8a. A solution of 0.25 mL (1.59 mmol) of diethyl (isocyanomethyl)phosphonate in 30 mL of anhydrous diethyl ether was placed in a flame-dried round-bottom flask under an atmosphere of nitrogen. The solution was cooled to −60 °C, and a 2.5 M solution of *n*-butyllithium in hexanes (0.7 mL, 1.76 mmol) was added in a dropwise manner with stirring. The resulting solution was stirred at −60 °C for an additional 15 min, and a solution of **4** (735 mg, 1.59 mmol) in 30 mL of dry diethyl ether was added via syringe. The cooling bath was removed, and the reaction mixture was allowed to warm to room temperature. Stirring at room temperature was continued until TLC showed that all of the starting material had reacted (48 h). Concentrated hydrochloric acid (2.4 mL) was then carefully added, and the mixture was vigorously stirred for 24 h. After the time period, cold water was added, the ether layer was separated, washed with water, and dried (Na_2SO_4), and the solvent was removed by rotary evaporation to afford an approximately 3:1 mixture (judged from proton NMR) of equatorial (**7a**) and axial (**7b**) aldehydes. The two diastereomers were separated by column chromatography on silica gel (5% ethyl acetate–petroleum ether as eluent) to give 317 mg of **7a** and 102 mg of **7b** in 55% combined yield: NMR ($CDCl_3$) **7a** δ 9.54 (s, 1H), 7.45 (m, 5H), 6.44 (d, 2H), 5.06 (s, 2H), 3.57 (d, 1H), 1.40 (s, 3H), 1.23 (s, 6H), 1.06 (s, 3H), 0.86 (t, 3H); **7b** δ 9.49 (s, 1H), 7.4 (m, 5 H), 6.43 (d, 2H), 5.04 (s, 2H), 3.62 (d, 1H), 1.36 (s, 3H), 1.23 (s, 6H), 1.00 (s, 3H), 0.85 (t, 3H). Compound **7a** (42.6 mg, 0.089 mmol) and 10 mg of palladium on active carbon in 7 mL of absolute ethanol were shaken under hydrogen (40 psi) at room temperature for 5 h. Then the catalyst was filtered off and the filtrate concentrated under vacuum. The residue was purified by column chromatography on silica gel (30% diethyl ether–petroleum ether as eluent) to afford 23.2 mg (68%) of compound **8a**: NMR ($CDCl_3$) δ 9.66 (s, 1H), 6.40 (s, 1H), 6.21 (s, 1H), 4.91 (s, 1H), 3.50 (d, 1H), 1.40 (s, 3H), 1.19 (s, 6H), 1.10 (s, 3H), 0.84 (t, 3H); HRMS calcd for $C_{25}H_{38}O_3$ 386.2821, found 386.2822.

Compound 8b. Compound **7b** (77 mg, 0.16 mmol) and 10 mg of 20% palladium hydroxide in 7 mL of absolute ethanol was shaken overnight under hydrogen (40 psi) at room temperature. The catalyst was filtered off, and the filtrate was concentrated under vacuum. The crude product was purified by column chromatography (20% ethyl ether–petroleum ether as eluent) to afford 25 mg (40%) of **8b**: NMR ($CDCl_3$) δ 9.86 (s, 1H), 6.35 (d, 1H), 6.27 (d, 1H), 5.07 (s, 1H), 3.56 (d, 1H), 1.39 (s, 3H), 1.20 (s, 6H), 0.99 (s, 3H), 0.84 (t, 3H); HRMS calcd for $C_{25}H_{38}O_3$ 386.2821, found 386.2817.

Reduction of Compound 8b. A 1 M solution of lithium tri-*tert*-butoxyaluminohydride in THF (45 μL, 0.045 mmol) was added to a solution of 16 mg (0.041 mmol) of **8b** in 7 mL of dry THF at 0 °C under nitrogen. The reaction mixture was stirred at 0 °C for about an hour. After the time period, the reaction was quenched by addition of water and the reaction mixture was concentrated under vacuum. The residue was dissolved in diethyl ether, the solution was washed with saturated brine until neutral and dried (Na_2SO_4), and the solvent was removed. Column chromatography on silica gel (80% diethyl ether–petroleum ether) afforded 11 mg (72%) of **2**. HPLC analysis on a Lichrosorb SI-60 (10 mm × 250 mm) column using 7% 2-propanol–hexane as eluent gave a single peak at a retention time of 19 min: NMR ($CDCl_3$) δ 6.31 (d, 1H), 6.23 (d, 1H), 3.75 (m, 2H), 2.22 (d, 1H), 2.47 (t, 1H), 1.33 (s, 3H), 1.19 (s, 6H), 1.06 (s, 3H), 0.84 (t, 3H); HRMS calcd for $C_{25}H_{40}O_3$ 388.2977, found 388.2981.

Reduction of Compound 8a. Equatorial aldehyde **8a** was reduced to alcohol 1 in 79% yield under identical reaction conditions described above for **8b**. HPLC analysis on a Lichrosorb SI-60 (10 mm × 250 mm) column using 7% 2-propanol–hexane as eluent gave a single peak at a retention time of 33 min: NMR ($CDCl_3$) δ 6.35 (s, 1H), 6.20 (s, 1H), 5.25 (s, 1H), 3.52 (d, 2H), 3.21 (d, 1H), 2.46 (m, 1H), 1.38 (s, 3H), 1.19 (s, 6H), 1.08 (s, 3H); HRMS calcd for $C_{25}H_{40}O_3$ 388.2977, found 388.2975.

Resolution of (±)-1. About 70 mg of (±)-1 was resolved by HPLC equipped with a semipreparative (250 mm × 20 mm) Chiralcel OD column using 4% 2-propanol in hexane as a mobile phase at a flow rate of 2 mL/min. The fractions were detected by UV (λ = 254 nm). Retention times of (−)-1 and (+)-1, under these conditions, were 32.0 and 35.4 min, respectively. About 27 mg of (−)-1 [mp 79.5−81.0 °C; $[\alpha]^{25}_D$ = −88.5° (*c* 1.2, $CHCl_3$) (lit.[2] mp 80−82 °C, $[\alpha]^{25}_D$ = −92°)] and 30.3 mg of (+)-1 [mp 79−81 °C; $[\alpha]^{25}_D$ = +88.5° (*c* 0.67, $CHCl_3$)] were obtained.

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