SYNTHESIS OF NONA-DEUTERO OLIVETOL AND NONA-DEUTERO CANNABINOIDS

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SUMMARY

Copper (I) catalyzed Grignard cross coupling of alkyl bromides provided a facile synthesis of d_9-olivetol [5-(2,2,3,3,4,4,5,5,5-$^2H_9$)pentyl-1,3-benzenediol] with high d_9 incorporation levels and no detectable levels of d_0 to d_7 ions. The outcome of the coupling is dependent on which bromide is used as the Grignard reagent. d_9-Δ^9-THC and racemic d_9-11-hydroxy-Δ^9-THC suitable for GC/MS analysis were prepared from the olivetol.

Key Words: Deuterium, Olivetol, Cannabinoids, Grignard, ^1H NMR, GC/MS

INTRODUCTION

Deuterium substituted Δ^9-THC and 11-hydroxy-Δ^9-THC were required as mass spectral internal standards for quantitation of the corresponding marihuana constitutent and its metabolite. In these studies, the analytes were the unsubstituted cannabinoid and metabolite and their tri-deutero analogs (1) which were administered to humans. The deuterated internal standards should have an incorporation of six or more deuterium atoms to preclude interference of the M-1 and M-2 ions of the standard with the M^+ ion of the d_3-analytes. Also, the absence of lesser deuterated components is desired as they would contribute interfering ions.

The sites of incorporation could not be at mass spectrally unstable positions. For example, preparation of a deutero-11-hydroxy-Δ^9-THC that includes two deuterium atoms in the 11-position, would not afford a viable standard since the loss of the 11-carbon is the major fragmentation pathway in the mass spectrum (MS) of the alcohol. Therefore, the mass spectrally stable pentyl side chain of the cannabinoids was chosen as the site of incorporation of the required deuterium atoms.

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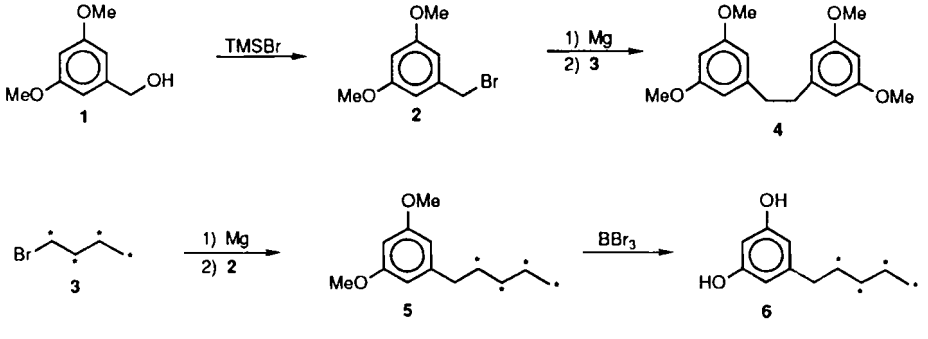
The pentyl side chain derives from olivetol which is a precursor of most chemical syntheses of cannabinoids (2). Thus, we sought to prepare the required deuterium substituted olivetol with high levels of the highest mass ion and negligible amounts of the unsubstituted, tri-deutero and other low mass ions which would interfere with the analyses. A d_7-olivetol derivative has been reported (3) from a synthesis that employed catalytic hydrogenation to introduce four of the seven deuterium atoms. This material had significant proportions of all levels of deuterium incorporation from d_7 to d_0 ranging from 66% to 1% respectively. Because of this difficulty with catalytic hydrogenation in achieving a high level incorporation and because of its potential in causing scrambling, we employed an approach that did not require catalytic hydrogenation at either the incorporation or subsequent steps.

RESULTS AND DISCUSSION

The approach we examined was the copper(I) catalyzed Grignard cross coupling of commercially available nona-deutero-1-bromobutane (3) with 3,5-dimethoxybenzyl bromide (2) (Figure 1). The method of cross coupling was developed for alkyl bromides (4) and has been applied in a synthesis of a d_3-olivetol (5) and dehydroolivetol (6). The benzyl bromide 2 (7) was prepared from commercially available 3,5-dimethoxybenzyl alcohol (1) via bromination with trimethylsilyl bromide (8). Formally, either bromide might be chosen as the Grignard component. The first choice employed the Grignard reagent from the benzyl derivative so as to minimize the number of transformations on the



*Labeled positions

Figure 1. Synthesis of Nona-deuteroolivetol (6)

costly 3. The reaction afforded none of the sought dimethoxyolivetol 5 when examined with unsubstituted 3 (GC). The major product (72%) was identified as the homo coupling product 4 by ^1H NMR and MS analyses. A minor product (7.5%) was identified as dimethoxytoluene. A report (6) of a similar cross coupling of the benzyl Grignard of 2 also generated 4.

The reverse coupling scheme was more successful. Treatment of unsubstituted 3 with 1.5 equivalents of magnesium afforded the corresponding Grignard reagent in 78% yield as determined by titration (9). The Grignard solution was treated with 2 and a catalytic amount of Li_2CuCl_4 to give 5 (66%) and the homo coupling product 4 (28%) after chromatography. The copper (I) promoted coupling of 2 with n-butyllithium gave similar results (7). The Grignard procedure was repeated on deuterium substituted 3 to afford 5 which was converted in 95% yield to deuterium substituted olivetol 6 by treatment with BBr_3 (1). Products 5 and 6 were identified by ^1H NMR and MS analyses. The latter was used to determine that the deuterium incorporation on the molecular ion cluster of 6 was 93% d_9, 7% d_8 and 0% of other ions.

The substituted olivetol was employed for the synthesis of the corresponding nona-deutero-Δ^9-THC (8, Figure 2). Thus, d_9-olivetol and p-mentha-2,8-dien-1-ol (7) were condensed and cyclized with $BF_3 \cdot Et_2O$ following the Razdan method (10). The resulting pure, pale resin was identical to authentic Δ^9-THC by TLC and GC. The ^1H NMR was consistent with that of unlabelled Δ^9-THC but with the absence of resonances for the β,γ,δ and ϵ protons and the collapse of the α-CH_2 triplet, seen in the unlabelled compound, to a singlet. The ^{13}C NMR spectrum agreed with the literature (11) with the notable difference that the resonances for the deuterated carbons were completely absent due to the much less effective relaxation of carbon by deuterium than

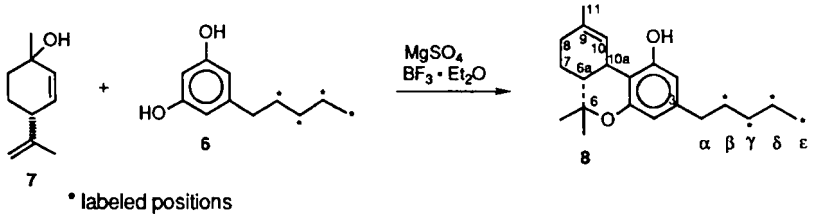
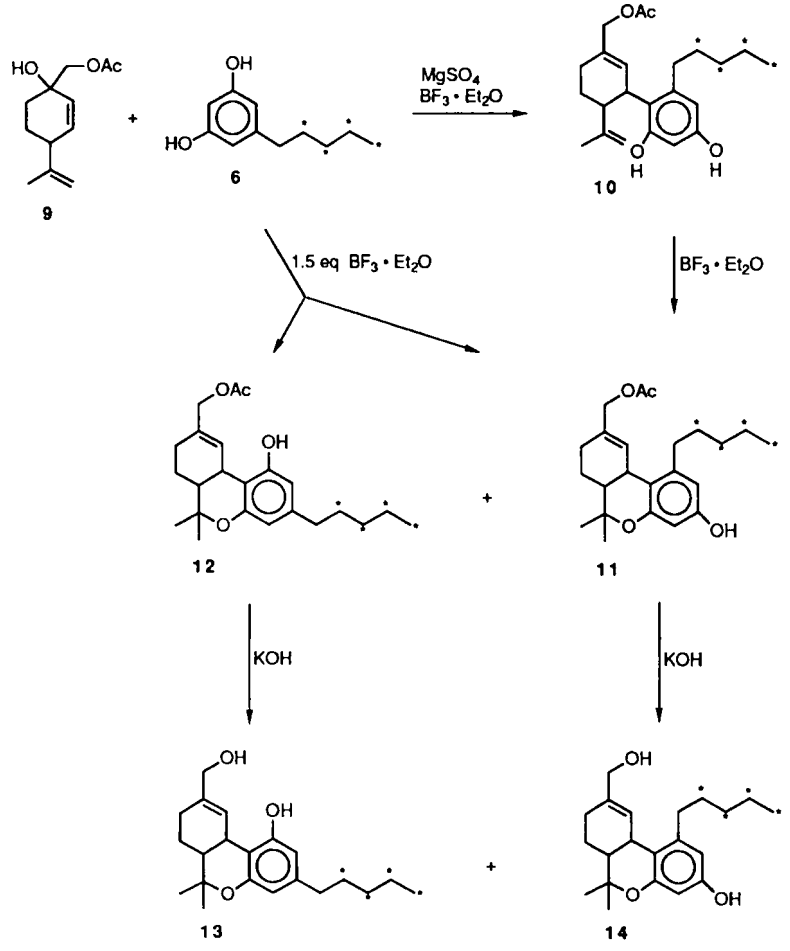


Figure 2. Synthesis of Nona-deutero-Δ^9-THC (8)

by proton (12). The mass spectrum demonstrated a fragmentation pattern consistent with a nona-deuterated side chain. The deuterium incorporation was determined from the m/z 323 cluster to be 93% d^9, 7% d^8 and 0% of other ions.

Racemic d_9-11-hydroxy-Δ^9-THC (13) was synthesized following a recently reported method for unsubstituted 11-hydroxy-Δ^9-THC (13) that allows incorporation of the d_9-olivetol late in the synthesis. The synthesis of the terpene 9 and its condensation with olivetol were reported without details. The diasteriomeric 9 was prepared from racemic perillaldehyde in four steps (13). We observed difficulties with regiospecificity in the condensation step which we report here with experimental details (Figure 3). The terpene and



• ^1H or ^2H sites

Figure 3. Preparation of Racemic D_9-11-Hydroxy-Δ^9-THC (13)

unsubstituted olivetol 6 were treated with $BF_3 \cdot Et_2O$ under standard Razdan conditions (10). The product of this reaction was (10) which when further treated with $BF_3 \cdot Et_2O$ afforded (11), an isomer of the sought 11-hydroxy-Δ^9-THC acetate (12). These compounds were identified by ^1H NMR, MS and saponification to a material that was not 11-hydroxy-Δ^9-THC (TLC) (14). Key to the recognition of the structure 14 was the upfield shifts in the ^1H NMR spectrum for the C-10 vinyl hydrogen and the C-10a hydrogen versus those for 13, which likely results from the steric influence of the nearby α-CH_2 (10). At the cannabidiol stage (10), the MS exhibited a base ion of m/z 244 which is explicable as deriving from 10 via a retro-Diels-Alder reaction followed by cyclization which eliminates acetic acid as shown in Figure 4.

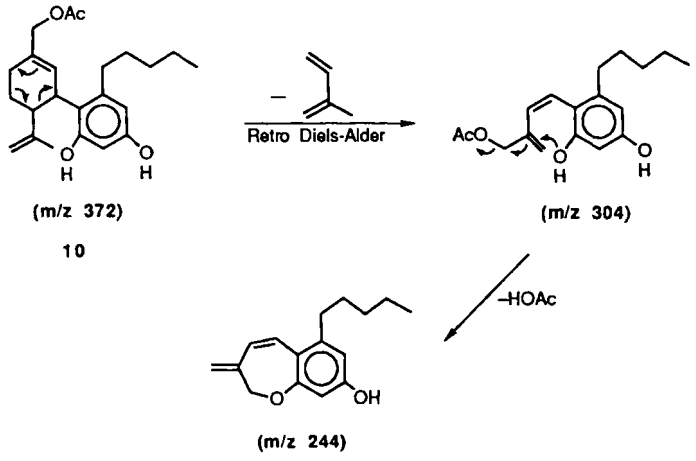


Figure 4. Mass Spectral Fragmentation of the Acetoxy Cannabidiol Isomer (10)

Modification of the reaction conditions (absence of $MgSO_4$, 1.5 eq $BF_3 \cdot Et_2O$, CH_2Cl_2, 2 h, 0°C) afforded approximately equal amounts of the 11-hydroxy-Δ^9-THC acetate isomers 12 and 11, which were readily separated by chromatography. The reaction was repeated with dg-olivetol to afford 12 and 11 in 17% and 21% yields, respectively. The acetate 12 was saponified and the resulting material was chromatographed to give 13 in 48% yield (8% overall from olivetol). This material was identical to authentic, undeuterated (-)-13 by GC (silylated sample) and TLC. ^1H NMR spectrum agreed with that of undeuterated (-)-13 with the expected differences of the absence of resonances

for the β,γ,δ and ϵ protons and the collapse of the α-CH_2 triplet to a singlet. The mass spectrum of a silylated sample indicated a deuterium incorporation of 84% d_9, 14% d_8, 1% d_7 and 1% d_{11} on the M-CH_2OSiMe_3 ion cluster; similar results were obtained on the unsilylated material for the M-CH_2OH ion cluster.

The above work provides a facile synthesis of deuterium substituted olivetol and cannabinoid standards with high levels of the d_9 standard peak, but without incorporation of lower mass components that would interfere with mass spectral analyses.

EXPERIMENTAL

^1H NMR spectra were obtained on a Varian EM390 or Bruker AM 250 spectrometer and the positive ion electron impact mass spectra were run on an AEI MS902 or GC HP 5890/MS HP 5988A mass spectrometer. Deuterium substituted n-butyl bromide was obtained from MSD Isotopes. TLC was performedo n Whatman silica gel 60A K6F plates. GC involved a 2% OV-17 column with flame ionization detection.

3,5-Dimethoxybenzyl bromide (2) (7)

3,5-Dimethoxybenzyl alcohol (1) (10.0 g, 59.5 mmol) was dissolved in 125 mL of CH_2Cl_2 in a dry three necked round bottom flask. To the resulting clear solution was added TMSBr (36.4 g, 238 mmol) dropwise at room temperature. The resulting light pink solution was stirred at room temperature for 16 h. GC and TLC showed the reaction to be complete. The solution was quenched with saturated aqueous $NaHCO_3$; $NaHSO_3$ was added and the solution was extracted with CH_2Cl_2 (5 x 100 mL). The combined organic extract was washed with saturated NaCl. Filtration of the CH_2Cl_2 extract through SiO_2, concentration and drying in vacuo gave white solid, 13.3 g (97%), GC (2% OV-17, 150 °C), rt. (retention time)(% of total peak area), 8.0 min (99.6%). ^1H NMR-90 MHz ($CDCl_3$) δ 3.82 (s, 6H, OMe), 4.43 (s, 2H, CH_2Br), 6.42 (d, 1H, J = 3 Hz, para-ArH), 6.55 (d, 2H, J = 3 Hz, ortho-ArH_2). HRMS: m/z Calcd: 229.9942, Found: 229.9940.

d_0- and d_9-Dimethoxyolivetol, [1,3-dimethoxy-5-pentylbenzene] (5)

Unlabelled n-butyl bromide (3) (0.75 g, 5.5 mmol) was added dropwise to Mg (0.20 g, 8.2 mmol, dried in oven for 3 h) and dry THF (2 mL) at ambient temperature. The reaction flask became hot as the Grignard reaction proceeded. When the flask cooled to room temperature, 3.5 mL THF was added and the reaction mixture was heated at reflux for 30 min. Titration (9) of the Grignard reagent with 2-propanol in presence of 1,10-phenanthroline showed it to be 0.8 M. When cooled, the Grignard was transferred to a Schlenk flask via cannula with 12 mL of THF. Benzyl bromide (2) (1.0 g, 4.4 mmol) was added as a THF solution (2 x 6 mL) at room temperature. The solution was cooled to ice bath temperature and dilithium tetrachlorocuprate (0.15 mL, 0.015 mmol) was added. The resulting mixture was stirred at 0°C for 3 h. GC and TLC showed the complete consumption of benzyl bromide. The reaction mixture was quenched with 2% H_2SO_4. THF was removed on rotary evaporator and the aqueous acid solution was extracted with CH_2Cl_2 until the extract showed no product by GC and TLC. The combined organic extract was washed with saturated NaCl, dried over $MgSO_4$, filtered, concentrated and dried in vacuo to give 0.90 g (98%) of the crude product. Chromatography on silica using 10% CH_2Cl_2/hexane gave 0.61 g (66%) of the desired product slightly contaminated with 3,5-dimethoxytoluene. GC (2% OV-17, 100-250°C, 10°/min, then 5 min at 250°C), rt. (% of total peak area), 8.5 min (94%), 4.4 min (4.1%). ^{1}H NMR-90 MHz ($CDCl_3$) δ 1.03 (t, 3H, J = 6.4 Hz, ϵ-CH_3), 1.3 (m, 4H, γ and δ-CH_2), 1.5 (m, 2H, β-CH_2), 2.62 (t, 2H, J = 8 Hz, α-CH_2), 3.82 (s, 6H, OCH_3), 6.35 (m, 3H, ArH_3). The product (C4) of homocoupling of 3,5-dimethoxybenzyl bromide was also obtained: 0.18 g (28%); ^{1}H NMR-90 MHz ($CDCl_3$) δ 2.91 (s, 4H, $(CH_2)_2Ar$), 3.81 (s, 12H, OCH_3), 6.40 (s, 6H, ArH_6). HRMS: m/z Calc.: 302.1519, Found: 302.1519.

When run with deuterium substituted n-butyl bromide (3) (4.81 g; 32.9 mmol), a yield of 3 g (54%) of dimethoxyolivetol was obtained; GC (2% OV-17, 100-250°, 10°/min, then 5 min at 250°), rt. (% of total peak area) 8.4 min, (98.6%). ^{1}H NMR-90 MHz ($CDCl_3$) δ 2.55 (s, 2H, α-CH_2), 3.78 (s, 6H, OCH_3), 6.33 (m, 3H, ArH_3). MS: m/z 217 (d_9, 92%), 216 (d_8, 8%).

d_9-Olivetol, [5-(2,2,3,3,4,4,5,5,5-$^2H_9$)pentyl-1,3-benzenediol] (6)

d_9-3,5-Dimethoxyolivetol (5) (3.0 g, 14 mmol) was dissolved in 80 mL of dry CH_2Cl_2. The clear solution was cooled to -78°C and treated with boron tribromide (7.0 g, 28 mmol) in 40 mL of CH_2Cl_2 added dropwise over a period of 1 h. After an additional 15 min, the flask was warmed to 0°C in an ice bath and the solution was stirred for 3 h. The resulting reaction mixture was left at room temperature over the weekend. The solution was poured into 300 mL of 10% $NaHSO_3$ containing ice and stirred. The aqueous layer was extracted with CH_2Cl_2 (3 x 200 mL) and ether (2 x 200 mL). The combined organic extract was washed with aqueous $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated to dry in vacuo to give 2.9 g of crude product. Chromatography on silica gel using 5% acetone/toluene provided 2.5 g (95%) of pure product: GC (2% OV-17, 180°C) rt. 5.1 min, 99.8%. ^1H NMR-90 MHz ($CDCl_3$) δ 2.5 (s, 2H, α-CH_2), 5.1 (br s, 2H, OH), 6.18 (d, 1H, J=1.5 Hz, C2-H), 6.24 (d, 2H, J=1.5 Hz, C4-H, C6-H). MS: m/z 189 (d_9, 93%), 188 (d_8, 7%).

d_9-Δ^9-THC (8)

^1H NMR-250 mHz ($CDCl_3$) δ 1.09 (s, 3H, 6α-Me), 1.41 (s, 4H, 6β-Me, 7-H), 1.68 (br s & dt, 4H, J = 2.2 Hz, 13 Hz, 9-Me & 6a-H), 1.90 (m, 1H, 7-H'), 2.17 (m, 2H, 8-H_2), 2.41 (s, 2H, α-CH_2), 3.20 (br d, 1H, J = 11.0 Hz, 10a-H), 4.70 (s, 1H, OH), 6.14 (d, 1H, J = 1.6 Hz, C2-H), 6.26 (d, 1H, J = 1.6 Hz, C4-H), 6.30 (br s, 1H, 10-H). ^{13}C NMR ($CDCl_3$) 1 sec relaxation time, δ/carbon: 154.6 (C1), 107.4 (C2), 142.4 (C3), 109.8 (C4), 154.1 (C4a), 108.9 (C10b), 77.1 (C6), 45.7 (C6a), 24.9 (C7), 31.0 (C8), 134.2 (C9), 123.6 (C10), 33.4 (C10a), 19.1 (6α-Me), 27.4 (6β-Me), 23.2 (9-Me), 35.1 (αC), not observed (βC, γC, δC, ϵC). MS: m/z 323 (base peak), 308, 280, 259, 243; 323 (d_9, 93%), 322 (d_8, 7%).

Racemic d_9-11-Hydroxy-Δ^9-THC (13)

A solution of d_9-olivetol (6) (500 mg, 2.65 mmol) and p-mentha-7-acetoxy-2,8-dien-1-ol (13) (9) (559 mg, 2.65 mmol) in 30 mL CH_2Cl_2 was treated with $BF_3 \cdot Et_2O$ (565 mg, 3.98 mmol) at 0°C with stirring under nitrogen. Upon completion (2 h) (TLC: 50% Et_2O/ CH_2Cl_2 and GC: 150 - 250°C, 5°/min) the reaction was quenched with solid $NaHCO_3$ and stirred at room temperature overnight. Filtration and evaporation of solvent afforded the product mixture

which was chromatographed on silica gel (Merck Lobar) eluting with CH_2Cl_2 to yield 174 mg (17%) of racemic d_9-11-acetoxy-Δ^9-THC (12): TLC (5% Et_2O/CH_2Cl_2) R_f = 0.5, ^1H NMR (90 mHz, $CDCl_3$) δ 1.14 (s, 3H, 6α-Me), 1.44 (s, 6β-Me), 2.09 (s, OAc), 2.41 (br s, α-CH_2), 3.28 (bd, 1H, 9 Hz, 10a-H), 4.49 (br s, 2H, 9-CH_2O-), 5.74 (s, 1H, D_2O exchangeable, phenol OH), 6.14 (br s, 1H, ArH), 6.24 (br s, 1H, ArH'), 6.82 (br s, 1H, 10-H), MS m/z 381. Further elution with 5% Et_2O/CH_2Cl_2 produced 209 mg (21%) of the d_9-11-acetoxy-Δ^9-THC isomer (11): R_f = 0.4, ^1H NMR-90 mHz ($CDCl_3$) δ 1.06 (s, 3H, 6-Me), 1.39 (s, 3H, 6-Me'), 2.06 (s, 3H, OAc), 2.57 (s, 2H, α-CH_2), 3.19 (br d, 1H, J = 9 Hz, 10a-H), 4.47 (s, 2H, 9-CH_2O), 6.06, 6.10 (br overlapping s, 2H, 10-H, OH), 6.14 (d, 1H, J = 3 Hz, ArH), 6.32 (d, 1H, J = 2 Hz, ArH').

To a degassed solution of d_9-11-acetoxy-Δ^9-THC (12) (170 mg, 0.446 mmol) in 22 mL methanol was added aqueous KOH (2.1 mL, 1:2 w:v solution) under N_2 at ambient temperature. At 15 h the volatile organics were removed in vacuo, the remained acidified to pH 5 with 1N HCl and partitioned between ether and water. The organic layer was dried over Na_2SO_4 and the residue upon removal of solvent in vacuo was chromatographed on silica gel eluting with 7.5% acetone/toluene to afford 73 mg of the title compound (13) (48%) as a solid. TLC: 25% acetone/toluene, R_f = 0.46; ^1H NMR-250 mHz ($CDCl_3$) δ 1.11 (s, 3H, 6α-Me), 1.4 (m, overlap, 7-H), 1.42 (s, 3H, 6β-Me), 1.71 (dt, 1H, J = 2.1 Hz, 11.7 Hz, 6a-H), 2.00 (m, 1H, 7-H'), 2.29 (m, 2H, 8-H_2), 2.41 (s, 2H, α-CH_2), 3.26 (bd, 1H, J = 10.8 Hz, 10a-H), 4.03 (br s, 2H, 9-CH_2O), 4.96 (br s, 1H, D_2O exchangeable, phenol OH), 6.12 (d, 1H, J = 1.6 Hz, ArH), 6.27 (d, 1H, J = 1.6 Hz, ArH'), 6.68 (d, 1H, J = 1.5 Hz, 10-H); UV (ϵ_{283} = 1560 L/mole·cm, abs. EtOH); MS (bis-TMS derivative, m/z 380, $M^{+\cdot}$ - CH_2OSiMe_3), 382 (d_{11}, 1%), 380 (d_9, 84%), 379 (d_8, 14%), 378 (d_7, 1%). MS (unsilylated, m/z 308, $M^{+\cdot}$ - CH_2OH), 309 (d_{10}, 1%), 308 (d_9, 83%), 307 (d_8, 14%), 306 (d_7, 2%).

Similar saponification of (11) afforded the d_9-11-hydroxy-Δ^9-THC isomer (14): ^1H NMR-250 MHz ($CDCl_3$) δ 1.06 (s, 3H, 6-Me), 1.40 (s, 3H, 6-Me'), 1.70 (t, J = 12 Hz, 6a-H), 1.97 (br s, 1H, 7-H), 2.30 (m, 2H, 8-CH_2), 2.59 (s, 2H, α-CH_2), 3.16 (br d, 1H, J = 9.7 Hz, 10a-H), 4.02 (s, 2H, 9-CH_2O), 6.02 (s, 1H, 10-H), 6.14 (d, 1H, J = 2.6 Hz, ArH), 6.31 (d, 1H, J = 2.6 Hz, ArH').

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