Journal of Labelled Compounds and Radiopharmaceuticals
J. Labelled Cpd. Radiopharm. **43**, 655-662 (2000)

Synthesis of [$^2H_6$]-Labelled Metabolites of Cannabinoids

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SUMMARY

Four [$^2H_6$]-labelled metabolites of cannabinoids were synthesized for use as internal standards for GC/MS analysis. The hexadeutero-ketone **3**, prepared from ketal **1**, undergoes a Shapiro reaction to give (±)-6,6-[$^2H_6$]dimethyl-11-nor-9-carboxy-Δ^9-THC (**4a**) as the major product. The minor product, (±)-6,6-[$^2H_6$]dimethyl-11-nor-9-carboxy-Δ^8-THC (**4b**), was obtained after hydrolysis of its acetate methyl ester which was isolated using preparative HPLC. Reduction of **4a** gave (±)-6,6-[$^2H_6$]dimethyl-11-hydroxy-Δ^9-THC (**6**). (±)-6,6-[$^2H_6$]dimethyl-8β,11-dihydroxy-Δ^9-THC (**10**), was also prepared from **3** using a reported procedure for the non-labelled analog.

Key Word: (±)-6,6-[$^2H_6$]dimethyl-11-nor-9-carboxy-Δ^9-THC, (±)-6,6-[$^2H_6$]dimethyl-11-nor-9-carboxy-Δ^8-THC, (±)-6,6-[$^2H_6$]dimethyl-11-hydroxy-Δ^9-THC, and (±)-6,6-[$^2H_6$]dimethyl-8β,11-dihydroxy-Δ^9-THC.

INTRODUCTION

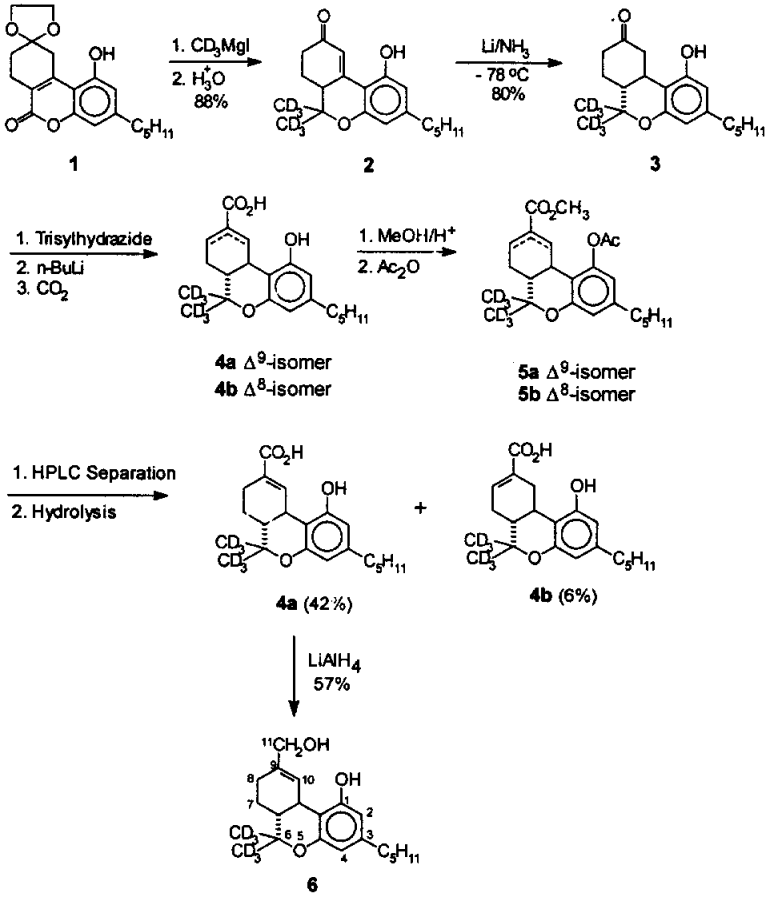
Δ^9-Tetrahydrocannabinol (Δ^9-THC) is the psychologically active component in the cannabis plant (marijuana) (1). Δ^9-THC is quickly metabolized and various metabolites have been found in human body fluids (2). The major metabolites include 11-nor-9-carboxy-Δ^9-THC, 11-hydroxy-Δ^9-THC and 8β,11-dihydroxy-Δ^9-THC and their glucuronide conjugates. GC/MS analysis of these metabolites confirms the illicit use of marijuana (3). The most suitable internal standards for GC/MS analysis are the deuterated derivatives of the drugs to be analyzed. Herein, the synthesis of hexadeutero-labelled analogs of the above mentioned metabolites, as well as a hexadeutero-labelled analog of 11-nor-9-carboxy-Δ^8-THC, the metabolite of a minor component (Δ^8-THC) of marijuana (2), is described.

The deuterium atoms of the deuterated derivatives described are positioned on the two geminal methyl groups on C-6 in the dibenzopyran structure. It was found that this location was superior to that with the deuterium atoms positioned on the terminal methyl group of the pentyl side chain in terms of the dynamic linearity ranges of analysis and ion ratios (4).

Received 16 October 1999
Accepted 13 January 2000

RESULTS AND DISCUSSION

Scheme 1 shows the synthetic route for (±)-6,6-[$^2H_6$]dimethyl-11-nor-9-carboxy-Δ^9-THC (**4a**), (±)-6,6-[$^2H_6$]dimethyl-11-nor-9-carboxy-Δ^8-THC (**4b**), and (±)-6,6-[$^2H_6$]dimethyl-11-hydroxy-Δ^9-THC (**6**). The precursor, **1**, prepared according to a reported procedure (5) from olivetol, was converted to **2** in 88% yield by treatment with C^2H_3MgI using THF as solvent. Birch reduction of **2** gave the (±)-hexadeutero-ketone **3** in a total yield of 80% after purification. The synthesis of **3** has been reported by ElSohly and Little (6), but in much lower yield. Kachensky and Hui (7) recently reported a one-pot synthesis of Δ^9-THC-9-COOH through a Shapiro (8) reaction from the non-deuterated analogue of **3**. Thus, **3** was treated with 2,4,6-triisopropylbenzenesulfonyl hydrazide (trisylhydrazide) to give a trisylhydrazone, which was subsequently converted to a vinyl lithium by treatment with *n*-butyl lithium. The intramolecular phenolic participation resulted in a kinetic deprotonation at C-10 and gave the $\Delta^{9,10}$ double bond as the major product. Quenching the vinyl lithium intermediate with CO_2 gave a mixture of **4a**

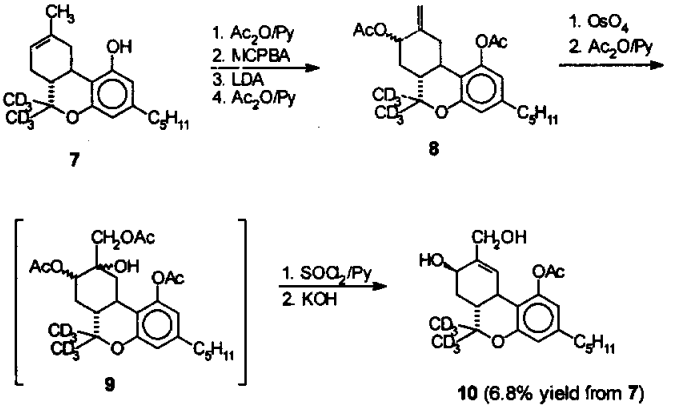


Scheme 1

(Δ^9-isomer) and **4b** (Δ^8-isomer) in about 85:15 ratio. Column chromatography followed by multiple recrystallizations gave pure **4a**. The mother liquor contained both **4a** and **4b** in about 1:1 ratio. Chromatographic separation of these two isomers proved rather difficult. However, we found that their derivatives (**5a** and **5b**) were readily separated by preparative HPLC under proper conditions. Pure **4a** and **4b** could be obtained by basic hydrolysis after HPLC separation. ElSohly and Little (6) also used **3** in the synthesis of **4b** through a 9-cyano intermediate. Treatment of **4a** with excess lithium aluminum hydride in THF gave (±)-6,6-[$^2H_6$]dimethyl-11-hydroxy-Δ^9-THC (**6**) in 57% yield.

As shown in Scheme 2, (±)-6,6-[$^2H_6$]dimethyl-8β,11-dihydroxy-Δ^9-THC (**10**) was synthesized from 6,6-[$^2H_6$]dimethyl-Δ^8-THC (**7**), which was obtained from **3** according to ElSohly and Little (6). The conversion of **7** to **10** followed a published procedure (9) for the synthesis of non-labelled 8β,11-dihydroxy-Δ^9-THC. Thus, epoxidation of the diacetate of **7** followed by isomerization with lithium diisopropylamide (LDA) and acetylation gave **8** as a mixture of epimers. Dihydroxylation of **8** with OsO_4 and then acetylation of the primary 11-hydroxyl group gave **9**, which was dehydrated with $SOCl_2$/pyridine and hydrolyzed to give **10** in 6.8% total yield from **7**. Direct oxidative functionalization at the allylic C-8 position of protected **4a** or **6** was attempted but was unsuccessful.

The isotopic purities for **4a**, **4b**, **6**, and **10** were determined to be greater than 99% by mass spectrometry (Figure 1). The structures of these compounds were also established by the ^{1}H-NMR spectra which were identical to those reported data (7, 9) of the corresponding unlabelled compounds, except for the total absence of the signals for dimethyl protons at C-6, indicating full deuteration of these methyl groups.



Scheme 2

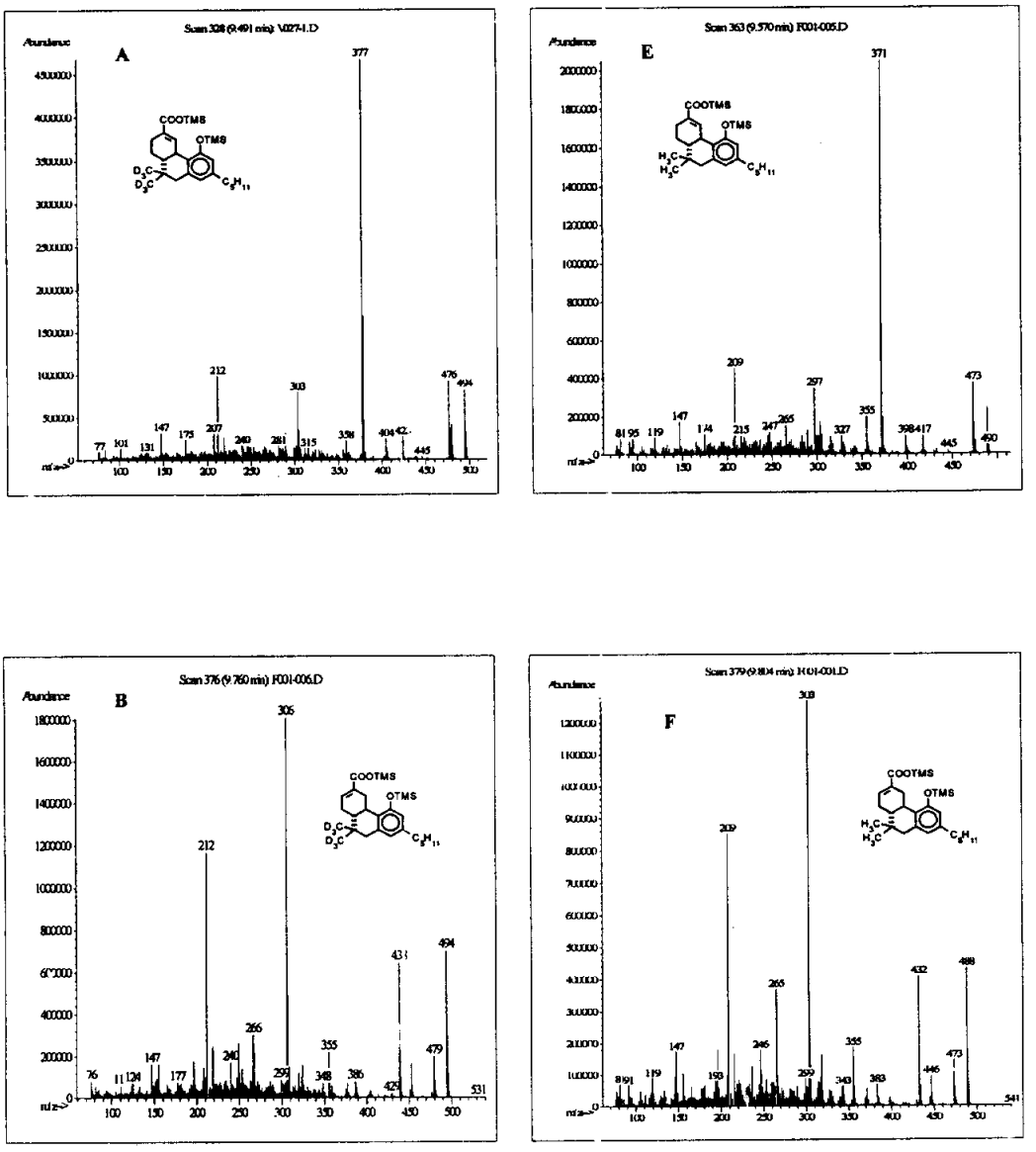


Figure 1. *(First section)* Mass spectra of the [$^2H_6$]-labelled metabolites of cannabinoids (A, B, C, and D), compared with reference spectra of non-labelled analogs (E, F, G, and H) as TMS derivatives.

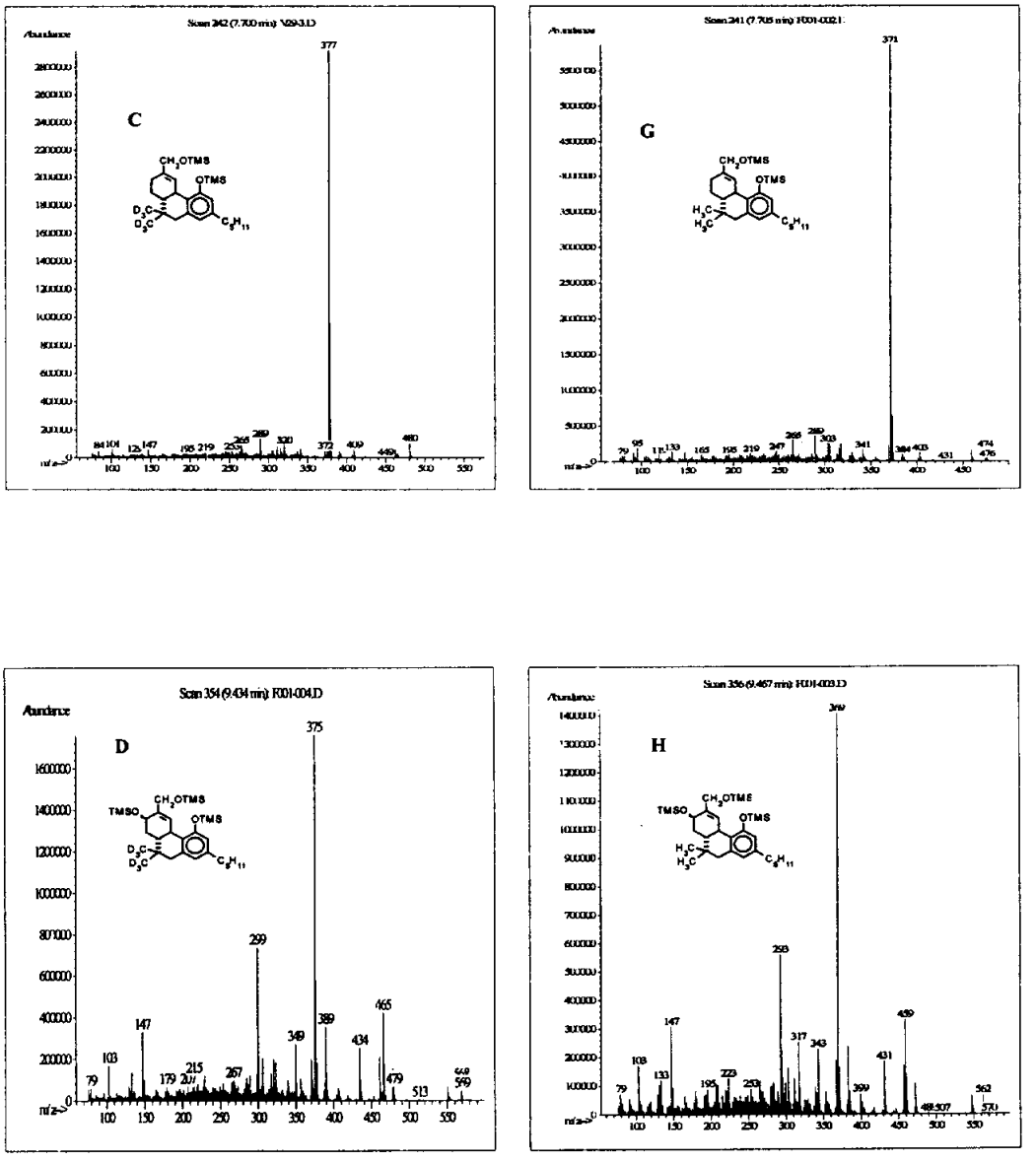


Figure 1. *(Second section)* Mass spectra of the [$^2H_6$]-labelled metabolites of cannabinoids (A, B, C, and D), compared with reference spectra of non-labelled analogs (E, F, G, and H) as TMS derivatives.

EXPERIMENTAL

NMR spectra were taken on a Bruker AC-300 spectrometer. GC/MS analyses were performed on a HP5890 gas chromatograph with a HP5970 mass selective detector in the E.I. mode (70 eV), equipped with a 25 m × 0.2 mm × 0.33 *u*m DB-5MS column. TMS derivatization was carried out by heating the analytes with excess bis(trimethylsilyl)trifluoroacetamide (BSTFA) in sealed GC vials. Reagents and dry solvents were purchased from Aldrich Chemical Company and used without further treatment.

(±)-6,6a,7,8-Tetrahydro-1-hydroxy-6,6-[$^2H_6$]dimethyl-3-pentyl-9*H*-dibenzo-[b,d]pyran-9-one (2): A solution of ketal **1** (8.30 g, 0.024 mole) in dry THF (120 mL) was added dropwise to C^2H_3MgI (99+ atom % D, 1M in ether, 240 mL, 0.24 mole) with vigorous stirring. The resulting yellow slurry was heated under refluxing temperature for two days. The mixture was cooled in an ice-bath and 60 mL of 1N HCl was added carefully to decompose the excess reagent. Then 80 mL of conc. HCl was added and the mixture was stirred vigorously for 1.5 h. Water (200 mL) was added and the mixture was extracted with ethyl acetate (3 × 100 mL). The organic layers were combined and washed with saturated $NaHCO_3$, water, and dried over Na_2SO_4. The solvent was removed *in vacuo* and the solid residue was triturated with hexane-ethyl acetate (3:2) and filtered to give 6.78 g (88% yield) of **2** as a pale-yellow crystalline solid: mp199-201°C [lit.(5) 198-199°C for unlabelled].

***trans*-(±)-6,6a,7,8,10,10a-Hexahydro-1-hydroxy-6,6-[$^2H_6$]dimethyl-3-pentyl-9*H*-dibenzo[b,d]pyran-9-one (3):** A solution of **2** (7.00 g, 0.022 mole) in dry THF (120 mL) was added dropwise with vigorous stirring to a mixture of liquid ammonia (250 mL) and lithium metal (0.25 g) in a dry ice-acetone bath. The addition was stopped whenever the blue color of the reaction mixture began to fade. More lithium metal was added and then the addition of **2** was resumed. This process was repeated until the blue color persisted for 10 min. Ammonium chloride was added until the blue color discharged and ammonia was allowed to evaporate at room temperature. Water (400 mL) was added and the pH was adjusted to 3 with conc. HCl. The mixture was extracted with methylene chloride (3 × 150 mL) and the combined organic layers were washed twice with brine, and dried over $MgSO_4$. The solvent was removed *in vacuo* to give a light brown oil which solidified upon addition of MeOH. This was recrystallized three times from MeOH-H_2O (7:1) to give pure **3** (4.09 g) as white needles: mp 149-150°C [lit. (5) 148-150°C; 163-165°C; and 162-165°C for non-labelled]. The mother liquor was purified by flash column chromatography (silica gel) eluting with hexane-ethyl acetate (85:15 to 80:20) which gave a second crop (1.54 g) of **3** (80% total yield): mp 150-151°C.

(±)-6,6-[$^2H_6$]dimethyl-11-nor-9-carboxy-Δ^9-THC (4a) and (±)-6,6-[$^2H_6$]dimethyl-11-nor-9-carboxy-Δ^8-THC (4b): **4a** was prepared from **3** in an analogous manner to that described by Kachensky and Hui (7) for the non-labelled Δ^9-THC-9-COOH. Ketone **3** (100 mg, 0.31

mmole) was treated with trisylhydrazide (94 mg, 0.32 mmole) to give the trisylhydrazone followed by *n*-butyl lithium (4.5 molar equivalent) at –78°C to 0°C and then CO_2 gas for 3 min. Work-up gave a residue which was subjected to column chromatography on silica (hexane:ethyl acetate 1:1) to give 54 mg of crude product (50% yield) as a white solid. HPLC analysis showed it was a mixture of **4a** and **4b** in 85:15 ratio. Recrystallization twice from ether-isooctane gave 36 mg of pure **4a** (>99.3% purity by HPLC): *m/z* (as di-TMS derivative): 494 ($M^{+\cdot}$, 28%), 479 (12%), 476 (27%), 377 (100%).

The mother liquid was concentrated to give an oil (27 mg) which was dissolved in 5 mL of methanol. Conc. H_2SO_4 (6 drops) was added carefully and the solution was heated under refluxing temperature for 4 hrs. The solvent was evaporated *in vacuo* and the product was partitioned between ethyl acetate and 5% $NaHCO_3$. The organic layer was evaporated to give an oil which was heated with acetic anhydride (0.5 mL) and pyridine (0.15 mL) at 80°C for 1 hr. The solvent was evaporated under a stream of nitrogen to give an oil (28 mg). This was dissolved in methanol (0.2 mL) and separated by semi-preparative HPLC using a 250 × 9.4 mm Spherisorb® C_{18} column eluted with acetonitrile:water:acetic acid (65:35:0.05) at a flow rate of 6.0 mL/min. Fractions containing **5b** (retention time = 18.4 min) and **5a** (retention time = 21.3 min) were collected. The solvent of each fraction were evaporated *in vacuo* and the residues were heated with 0.5 mL of methanol and 0.2 mL of 5N KOH at 50°C for 2 hrs. The pH of each fraction was adjusted to 4 with 2N HCl to precipitate the product. Each product (**4a** or **4b**) was collected by filtration. Yield of **4a** (9.4 mg, total of 45.4 mg was obtained) was 42% total. Yield of **4b** (6.5 mg) was 6%: *m/z* (as di-TMS derivative): 494 ($M^{+\cdot}$, 39%), 479 (11%), 452 (9%), 438 (37%), 306 (100%).

(±)-6,6-[$^2H_6$]dimethyl-11-hydroxy-Δ^9-THC (6): $LiAlH_4$ (70 mg) was added to a solution of **4a** (64 mg) in dry THF (5 mL). After the mixture was stirred at rt for 3 hrs, EtOAc followed by 0.1 N HCl was added carefully. The organic layer was separated and the aqueous layer was extracted with EtOAc once. The combined organic layers were washed once with H_2O, once with brine, and dried over $MgSO_4$. Pure **6** obtained after column chromatography on silica gel (hexane:EtOAc 1:1) as a white solid (35 mg, 57% yield): *m/z* (as di-TMS derivative): 480 ($M^{+\cdot}$, 5%), 377 (100%).

(±)-6,6-[$^2H_6$]dimethyl-8β,11-dihydroxy-Δ^9-THC (10): This compound was prepared from **7** in an identical manner as described by Pitt et al. (9) and was obtained as an off-white solid (6.8% total yield): *m/z* (as tri-TMS derivative): 568 ($M^{+\cdot}$, 5%), 465 (24%), 375 (100%).

ACKNOWLEDGMENT

This work was supported in part by the National Institute on Drug Abuse (NIDA), SBIR contract N44DA-7-7057.

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