Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXV, No. 1

SYNTHESIS OF DEUTERIUM LABELED CANNABINOIDS

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SUMMARY

Several methods for the specific deuteration of cannabinoids are described. Deuteration of the phenolic ring was accomplished by treatment with BF_3 .Et_2O followed by quenching with a solution of Na_2CO_3 in D_2O resulting in deuterium incorporation in both the 2 and 4 positions. Regioselective incorporation of deuterium into either the 2 or 4 position of Δ^8-THC was achieved using Florisil spiked with either D_2O or H_2O. Deuteration at positions 8, 10 and 11 was achieved by addition of DCl gas to the appropriate tetrahydrocannabinol to form 9-chlorohexahydrocannabinol labeled at either of the above positions, followed by elimination of hydrogen- or deuterium chloride with potassium-tert-amylate. UV irradiation of specifically labeled Δ^8 -THC gave the correspondingly labeled $\Delta^{9,11}$ -THC.

KEYWORDS: Deuterium labeled tetrahydrocannabinols, Δ^8 -THC, Δ^9-THC, $\Delta^{9,11}$ -THC, Cannabinol, Cannabidiol.

INTRODUCTION

Cannabis has been known since ancient times to elicit psychotomimetic responses in man. However, recent research efforts have focused on the therapeutic application of certain tetrahydrocannabinols (THC's), especially as analgetics , antiemetics and agents for the treatment of glaucoma (1). Our involvement in cannabinoid chemistry is connected with our interest in understanding the mechanism with which drug molecules affect cellular membranes (2,3). Indeed, pharmacologically active cannabinoids are thought to interact with membrane phospholipids in such a manner as to "perturb" the lipid bilayer, causing modulation of the function of different proteins imbedded in the bilayer (4-6).

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0362-4803/88/010073-10$05.00
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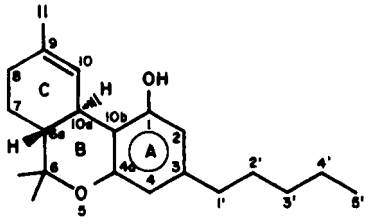
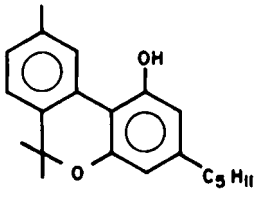
Received March 24, 1987
Revised May 13, 1987

Among the methodologies available for studying drug : membrane interactions, we found solid-state nuclear magnetic resonance (NMR) spectroscopy to be particularly useful. Recently, we became very interested in studying the orientation of cannabinoids in membrane bilayers. Such a study require the preparation of cannabinoids isotopically labeled with deuterium in selected positions of the molecule.

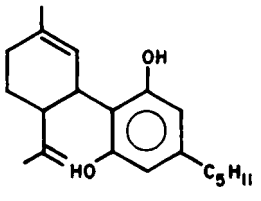
Since 1968, several methods for the selective incorporation of deuterium in tetrahydrocannabinoids have been described including labeling of the aromatic ring (7,8), the B and C rings (7-9) and the pentane side-chain of THC's (10).



Δ^9 - THC (1) with numbering scheme



Cannabinol (4)



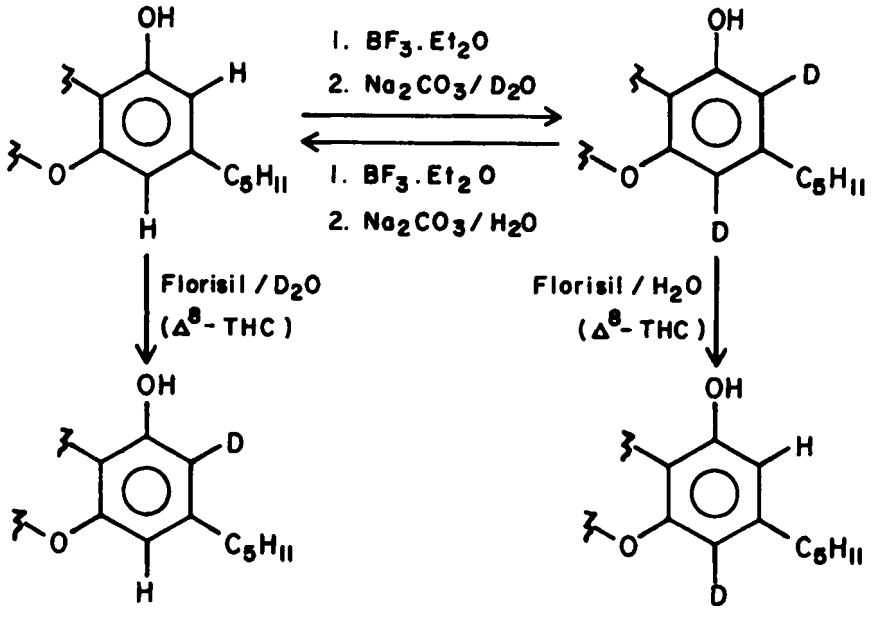
Cannabidiol

We wish to report on the synthesis of several cannabinoid analogs regiospecifically labeled with deuterium in different positions of the tricyclic ring system. Several of these deuterated analogs are reported here for the first time. Furthermore, we have developed a new method for the labeling of the aromatic ring (11) and made use of a reaction involving the addition and elimination of DCl on THC analogs as a means of labeling the C-ring.

RESULTS AND DISCUSSION

1. Deuteration in the aromatic ring

Deuteration at positions 2 and 4 of the phenolic ring was achieved by a simple and highly selective method which entails treatment of the compounds under mild conditions with $BF_3.Et_2O$ followed by quenching with a solution of sodium carbonate in D_2O. Δ^8-THC (2) could be deuterated at room temperature, while Δ^9-THC (1) $\Delta^{9,11}$-THC (3) and cannabidiol (CBD) partially isomerized to the more stable Δ^8-THC under these conditions (12,15). However, we were able to obtain excellent aromatic ring deuterium labeling of these analogs without isomerization by carrying out the reaction at $-30°C$ (Scheme 1). In the case of cannabinol (4), a molecule with two aromatic rings, deuteration occurred exclusively in the 2 and 4 positions of the phenolic ring. Yields for this reaction consistently ranged at 85-95% deuterium incorporation.

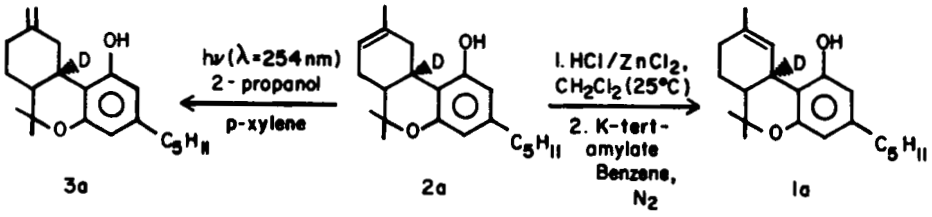


SCHEME 1

In addition to this method, we have found that Florisil (magnesium silicilate), in the presence of small amounts of D_2O can be used as a deuterium exchange catalyst for the introduction of deuterium in the aromatic ring of phenolic systems. Furthermore, we were able to use this method with Δ^8-THC (2) to obtain selective incorporation of deuterium in the 2 or 4 positions of the phenolic ring. This was of particular interest to us for our solid state ^{2} H NMR experiments where we needed to discriminate between the deuterium labels in each of these two positions. We found that the exchange at position 2 of the aromatic ring of Δ^8-THC proceeds rapidly while the hydrogen at position 4 hardly exchanges at all. This apparent selectivity provided us with a means of obtaining the desired products namely, Δ^8 -THC-2d by deuteration of Δ^8-THC with Florisil pretreated with D_2 O and Δ^8-THC-4d by selective dedeuteration of Δ^8-THC-2,4-d_2 using Florisil pretreated with H_2 O (Scheme 1). By using this method, we were able to obtain 70% D at position 4 versus 10% D at position 2 using the dedeuteration procedure, and 60% D at position 2 versus 20% D at position 4 with the deuteration procedure. Both reactions were carried out at room temperature. Dedeuteration proceeded at a much faster rate than deuteration, while the 2:4 isotope exchange ratio is also less favorable with deuteration than with dedeuteration.

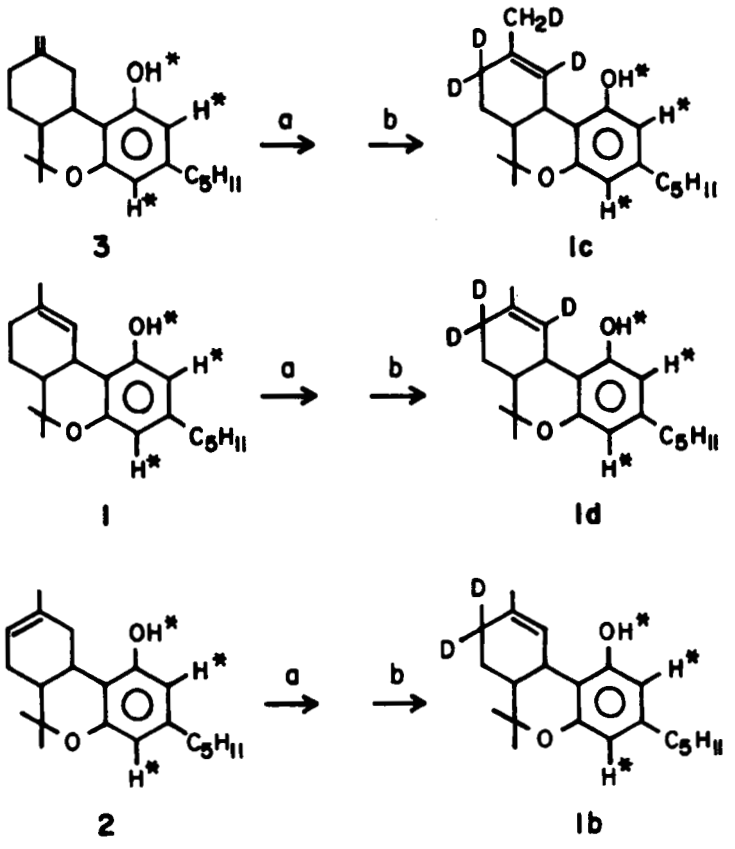
2. Deuteration in the B and C rings

(10a-d)-Δ^9-THC (1a) was prepared from the correspondingly labeled Δ^8- analog using a method developed by Mechoulam *et al.* (13) for the conversion of Δ^8 - to Δ^9-THC. The method involves addition of HCl gas to (10a-d)-Δ^8-THC (2a) followed by dehydrochlorination of the 9-chlorohexahydrocannabinol (HHC) intermediate with potassium-tert-amylate to yield (10a-d)-Δ^9-THC. We used the HCl addition/elimination reaction to introduce deuterium labels regiospecifically in the C-ring of Δ^9-THC (Scheme 2).



SCHEME 2

Δ^9-THC deuterated at the 8 position (C ring) was obtained by addition of DCl to the double bond of Δ^8 -THC and subsequent elimination of HCl by potassium-tert-amylate. When performed at 0°C, deuterium incorporation in the intermediate 9-chloro-HHC was found to be in both the 8α and 8β positions while no deuterium incorporation was found in the 10 position. This was confirmed by comparison of the ^{1}H NMR spectra of the deuterated intermediate with that of the undeuterated product obtained from HCl addition. The amount of deuterium incorporation at the 8α and 8β positions was approximately 50% in each of these positions and was retained during the elimination step to yield Δ^9-THC with ca. 50% D in each of the 8α and 8β positions (1b). Δ^9-THC labeled in the 8 and 10 or in the 8, 10 and 11 positions (1c) could be obtained through a similar sequence by passing DCl in a Δ^9 or $\Delta^{9,11}$-THC solution (Scheme 3).



a = DCl /$ZnCl_2$ in CH_2Cl_2 at 25° C

b = k - tert-amylate, Benzene / N_2

H^+= H or D

SCHEME 3

Deuterium NMR spectra of 1b and 1c confirmed the presence of deuterium in the positions indicated (Figures 1 and 2). Deuterium incorporation in the 8 position of the C ring presumably occurs because of the addition of DCl to the Δ^8 isomer which forms as an intermediate during the addition of DCl. We were indeed able to confirm the presence of Δ^8-THC as an intermediate in this reaction, using ^{1}H NMR.

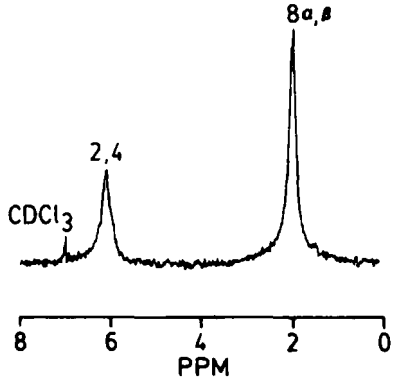


Figure 1. 55.2 MHz Deuterium NMR spectrum of compound (1b).

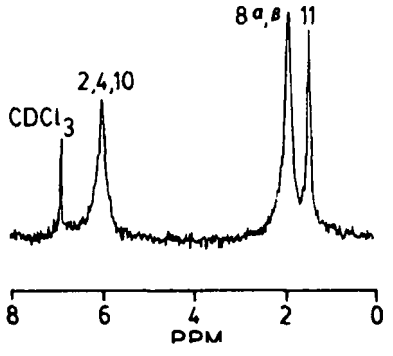
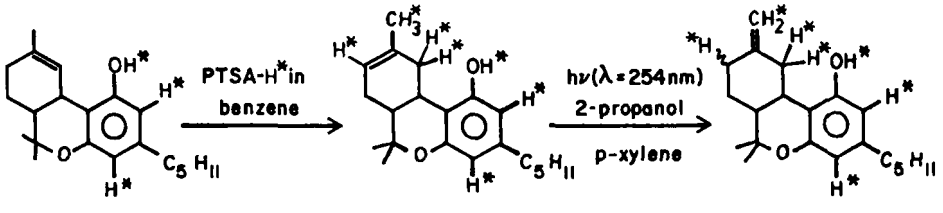


Figure 2. 55.2 MHz Deuterium NMR spectrum of compound (1c).

We found that deuterium incorporation could be maximized by DCl addition to tetrahydrocannabinols which had been deuterated in the 2 and 4 positions and the phenolic hydroxy prior to the reaction. When removal of the two aromatic deuteria was required, this could be accomplished by a simple dedeuteration using BF_3 .Et_2 O as described in the experimental section, and quenching with an aqueous sodium bicarbonate solution.

Stereospecific labeling at position 10β (axial) of Δ^8 -THC was attempted according to the method described by Burstein and Mechoulam (9) using deuterated p-toluene sulfonic acid to isomerize Δ^9 -THC to the deuterium labeled Δ^8 -THC. The ^{1}H NMR spectrum of the product showed that indeed isomerization had taken place; but in our hands deuteration occurred not only at 10β , but also at 10α, 8 and 11 (Scheme 4). The



PTSA= p-toluenesulfonic acid

H* = H or D

SCHEME 4

proton multiplets for H 10a in the non-deuterated Δ^8-THC consists of a triplet of doublets due to two large trans diaxial coupling, (^3J 10a,10β = 10 Hz; ^3J 10a,6a = 10.5 Hz) and one gauche axial equatorial coupling (^3J 10a,10α = 3.5 Hz). When both 10α and 10β are substituted with deuterium, the H 10a resonance collapses to a broad doublet (^3J 10a,6a = 10.5 Hz). The couplings of H 10a with the deuterium in the 10α and 10β positions are much smaller (approximately 15% of the ^{1}H couplings) and not easily observable, thus resulting in the broadening of the doublet. If only the 10α position is deuterated, the H 10a multiplet is now a triplet due to vicinal coupling with H 10β and H 6a. On the other hand if only 10β is deuterated the 10a multiplet is a broad doublet of doublets because of coupling with H 6a and H 10α. The observed H 10a signal in the ^{1}H NMR spectrum of the product is a superposition of all the above (Figure 3). A careful analysis indicated approximately 50% deuterium incorporation in each of the 10α and 10β positions. Integration of the H8- and 9-methyl signals at 5.43 and 1.7 ppm respectively showed deuterium incorporation of ca. 50% at 8 and some in the 11 position. These estimates and the positions of deuterium incorporation was confirmed from the ^{2}H NMR spectrum of the product (Figure 4).

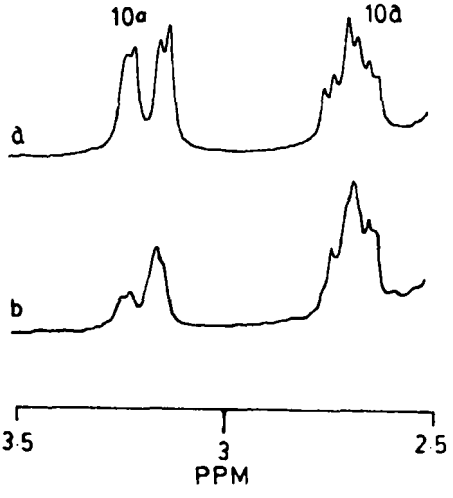


Figure 3. Partial proton NMR spectra of Δ^8-THC (a) and (2,4,8,10,11-d_6)-Δ^8-THC (b) showing the signals for the 10 α and 10a protons.

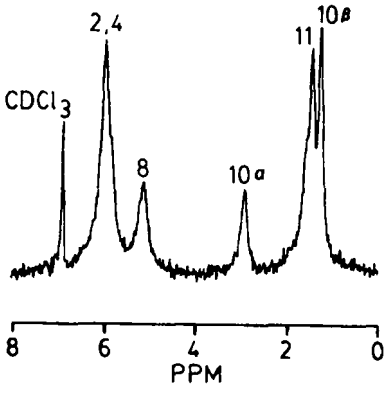


Figure 4. 55.2 MHz Deuterium NMR spectrum of (2,4,8,10,11-d_6)-Δ^8-THC.

$\Delta^{9,11}$-THC labeled at positions 10a (3a ,Scheme 2), 8 or 10 (Scheme 4) was prepared by UV irradiation of the correspondingly labeled Δ^8 -THC (14) using p-xylene as sensitizer. We found that cooling by means of an immersed cold finger avoided scrambling of the label and polymerization of the products.

EXPERIMENTAL

Proton NMR spectra were obtained on a 200 MHz IBM WP-200SY spectrometer using tetramethylsilane as internal reference. Deuterium NMR spectra were obtained at 55.2 MHz on the home-built 360 MHz spectrometer at the Francis Bitter National Magnet Laboratory, M.I.T., Cambridge, Massachusetts. Deuterium chloride gas was purchased from MSD Isotopes. All chemicals and chromatographic materials were purchased from Aldrich Chemical Co. Solvents involved in the experiments were dried using suitable methods prior to use. Glassware was flame dried.

The following are general methods for the preparation of labeled cannabinoids in the positions indicated.

1) Preparation of (2,4-d_2)-tetrahydrocannabinols

a- Using BF_3. Et_2O and Na_2CO_3 in D_2O

To a solution of 250 mg of the cannabinoid in 10 ml of dry methylene chloride, 1 ml of boron trifluoride etherate was added under nitrogen and the mixture stirred at $-30°C$ for a further 30 min. The reaction was then quenched with 5 ml of a solution of 10% sodium carbonate in D_2O and allowed to warm up to room temperature under stirring. The organic layer was washed with D_2O and dried over anhydrous Na_2SO_4. The solution was filtered and the solvent evaporated to give 250 mg (100%) of the deuterated compound. The percentage of deuterium incorporated was determined from ^1H NMR to be 90% at each of the 2 and 4 positions. Deuteration could be increased close to 100% by repeating this reaction. Quantitative dedeuteration of the deuterated products could be achieved by a repetition of the above procedure, by substituting D_2O with H_2O in the quenching and washing steps.

b- Using Magnesium Silicilate (Florisil) and D_2O

In a typical procedure for the regioselective labeling with deuterium in either the 2 or 4 position, 2.5 g of Florisil pre-dried at 350°C, was treated with either deuterium oxide or protium oxide (100-200 µl) and left to equilibrate for 2 hrs. A slurry was then prepared by stirring the Florisil into 20 ml of a 1% ether in petroleum ether solution and left to stir for 15 min. Undeuterated or deuterated Δ^8-THC (200 mg) was added to the D_2O and H_2O-containing mixtures, respectively, and stirring was allowed to continue in a sealed container. Samples, from which ^1H NMR spectra could be obtained in order to check the progress of the reaction, were drawn at specific intervals. The proton spectra of the deuterated products, as compared with that of their respective substrates, confirmed the regiospecificity of deuterium incorporation through marked reduction in the intensity of either the 2 or 4 aromatic ring proton resonances.

2) Preparation of 9-chloro-HHC as precursor for the preparation of (10a- d)-Δ^9-THC (1a), (2,4,8- d_4)-Δ^9-THC (1b), (2,4,8,11- d_5)-Δ^9-THC (1c) and (2,4,8,10- d_5)-Δ^9-THC (1d)

Dry DCl gas was added through a gas dispersion tube to a methylene chloride solution containing zinc chloride (200 mg) and an appropriate (2,4-d_2)-THC compound (500 mg) at 0°C. The addition was allowed to proceed under dry conditions for 1 hr, after which the mixture was allowed to warm to room temperature and stirred overnight. The mixture was first washed with a 10% Na_2CO_3 / D_2O solution (2x5 ml) and then with D_2O (2x5 ml) after which the organic layer was dried over anhydrous Na_2SO_4 and evaporated under vacuum to give the product in quantitative yield.

3) Elimination of HCl for the preparation of (10a-d)-Δ^9-THC (1a), (2,4,8- d_4)-Δ^9-THC (1b), (2,4,8,11- d_5)-Δ^9-THC (1c) and (2,4,8,10- d_5)-Δ^9-THC (1d) from the appropriately labeled 9-chloro-HHC

Appropriately labeled 9-chloro-HHC (900 mg) was dissolved in dry benzene (12 ml) and cooled to 0°C under a nitrogen atmosphere. Freshly prepared potassium-tert-amylate (8.3 ml of 1M solution in benzene) was added and the mixture stirred for 30 min. The mixture was then warmed to 70°C, stirred until the reaction was completed (3 hrs, TLC; 10% ether in petroleum ether), diluted with benzene and neutralized with dry ice to pH 7, after which it was extracted with ether (2x30 ml). The ether layer was washed with water (5 ml), aqueous $NaHCO_3$ (5 ml) and again with water (5 ml), after which it was dried over anhydrous Na_2SO_4 and evaporated under vacuum to yield the Δ^9-THC in quantitative yield.

4) Synthesis of (2,4,8,10,11- d_6)- Δ^8 -THC

A solution of (2,4- d_2)- Δ^9 -THC (100 mg), in which the phenolic H had been exchanged by exposure to D_2O was dissolved in dry benzene (100 ml). Deuterated p-toluene sulfonic acid was added and the mixture refluxed for 2 hrs after which it was washed with 5% Na_2CO_3 / D_2O (5 ml). The organic phase was separated, washed with D_2O (5 ml) and dried over anhydrous Na_2SO_4 . Evaporation gave (2,4,8,10,11- d_6)-Δ^8-THC in 75% yield (see Scheme 4).

5) Synthesis of (10a- d)-$\Delta^{9,11}$-THC (3a) and (2,4,8,10,11- d_6)-$\Delta^{9,11}$-THC

A solution of the appropriately labeled Δ^8-THC (300 mg) in a mixture of 2-propanol (180 ml) and p-xylene (2 g) in a quartz cylinder equipped with a cooling device (cold finger, circulating water at 5 – 10°C) was irradiated with a low pressure mercury lamp (Rayonet, 7.6 milli-einsteins/hr) under a nitrogen atmosphere until all starting material had been transformed (ca. 50 hrs). The solvent was then evaporated to yield the labeled $\Delta^{9,11}$ -THC compound in 30% overall yield.

6) Purification

Purification of all the above samples was accomplished by column chromatography on Florisil and elution with 0.5% ether in petroleum ether as mobile phase.

ACKNOWLEDGEMENTS

This research was supported by a grant from the National Institute on Drug Abuse (DA-3801) and the University of Connecticut Research Foundation (UCRF-35491). One of us (CJVdS) thanks the Foundation for Research Development, CSIR, Pretoria, for a sabbatical grant and Noristan Limited for support.

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15. We found that treatment of $\Delta^{9,11}$-THC with BF_3 at room temperature resulted in quantitative isomerization to Δ^8-THC.