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DEUTERATION AND TRITIATION OF Δ^8 AND Δ^9-TETRAHYDROCANNABINOL
THE USE OF TRIFLUOROACETIC ACID AS A CONVENIENT LABELLING REAGENT

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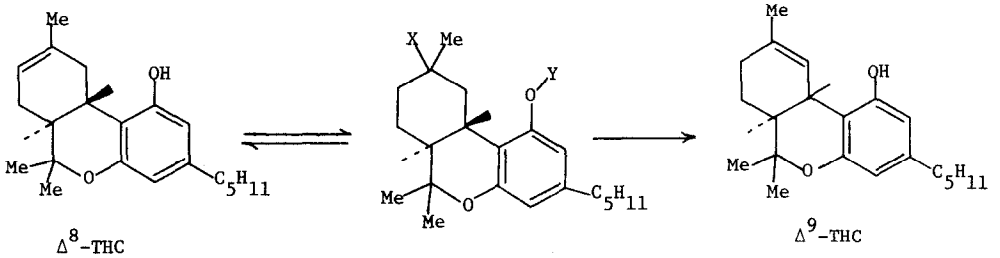
We should like to draw attention to some advantages of using trifluoroacetic acid as a reagent for deuteration and tritiation, with particular reference to the labelling of Δ^8- and Δ^9-tetrahydrocannabinol (THC). Compounds which exchange hydrogen in the presence of an acid catalyst are commonly labelled by treatment with protic acids such HCl or H_2SO_4 in an inert or deuterated solvent, or by treatment with Lewis acid-protic acid mixtures such as $AlCl_3$:H_2O or BF_3:H_3PO_4 (1). While these various reagents have their own specific advantages, trifluoroacetic acid possesses the following very practical assets.

Labelled trifluoroacetic acid is simply obtained, in quantitative yield, by mixing trifluoroacetic anhydride and labelled water; it has excellent solvent properties, and functions as both the acid catalyst and the source of label; it contains no superfluous protons and so the uptake of label incorporation may be conveniently following by nmr; it is volatile (bp 72.4°) and so is readily removed by evaporation in vacuo. In the case of olefins, hydrogen exchange and addition of the reagent to form the trifluoroacetate occur simultaneously. This addition reaction may be used to some advantage, as we now illustrate in the following example involving the synthesis of labelled Δ^8- and Δ^9-THC (Fig. 1).

Δ^8-THC (3.0 g) was dissolved in trifluoroacetic anhydride (3 ml) to affect conversion to the 1-trifluoroacetate, and remove the labile phenolic proton. After 1 hr, unchanged anhydride was evaporated in vacuo and the residual ester was redissolved in trifluoroacetic anhydride (7.5 ml, 51 mmole) and cooled to 0°. Tritium oxide (1.0 g, 55 mmole, 25 mCi) was added to the mixture, and the solution was stirred overnight at room temperature under nitrogen and then



Δ^8-THC

I, X, Y = O·CO·CF_3

II, X = O·CO·CF_3: Y = H

III, X = Cl: Y = H

Δ^9-THC

poured into water (150 ml).* The precipitated product was extracted with petroleum ether, washed with aqueous sodium bicarbonate and dried. Concentration afforded the 1,9-bis-trifluoroacetate (I); ν_{max}^{film} 1780, 1800 cm^{-1}; Calcd m/e for $C_{25}H_{30}F_6O_5$: 524.200; Found: 524.201. The bis-ester was dissolved in methanol (50 ml) and, after standing overnight, the methanol was evaporated to leave the 9-trifluoroacetate (II): ν_{max}^{film} 1780, 3400 cm^{-1}; Calcd m/e for $C_{23}H_{31}F_3O_4$: 428.217; Found: 428.217.

The 9-trifluoroacetate was heated in vacuo (0.2 mm) in a bulb to bulb distillation apparatus. Some distillation was observed at 100°. The bulk of the material (2.25 g, 75% based on starting Δ^8-THC) distilled at 200°-245° and was shown by infrared and glc analysis (2% OV-17 on gaschrom Q) to be a mixture of Δ^8-THC (84%) contaminated with Δ^9-THC (shoulder on Δ^8-THC peak, <5%), cannabidiol (4%)** and an unidentified impurity (10%) which was removed by elution from silica gel. The activity of the Δ^8-THC was 0.24 mc/mmole.

The location of the label was revealed by an analogous experiment with deuterium. Trifluoroacetic acid (100% d_1, 0.1 mole) and Δ^8-THC (9.55 mmole) were allowed to interact until nmr analysis showed the absence of the olefinic proton. The diester was isolated as described above, and then converted to deuterated II and to Δ^8-THC. The mass spectrometrically determined deuterium contents are recorded in Table 1. It is obvious that there are at least four acid-labile protons in the tetrahydrocannabinol molecule. The presence of deuterium on the aromatic ring is clearly shown by integration of the aromatic p.m.r. spectrum, and by the m/e 231

*Alternatively, if a means of removing radio-active vapor is available, the mixture is more conveniently worked up by concentrating in vacuo.

**Present in starting material.

TABLE 1

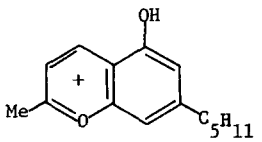
Percentage Deuterium Incorporation by Various Compounds in CF_3COOD

Compound	Reaction Time	% do	% d_1	% d_2	% d_3	% d_4	% d_5	Σ d per mole	Theor. Σ d[a]
II	18 hr	18	48	26	6	2	1	1.3	2.6[b]
m/e 231 from II	-	72	23	5	1	-	-	0.35	1.3
Δ^8-THC	-	42	40	11	4	2	-	-	-
m/e 231 from Δ^8-THC	-	76	19	3	2	-	-	-	-
Anisole	144 hr	-	1	41	58	-	-	2.6	2.25
2-Methylcyclohexanone	23 hr	-	23	40	37	-	-	2.1	2.25
Triethylcarbinol	24 hr	5	20	26	29	16	5	2.45	3.6

a) Maximum deuterium incorporation possible assuming complete equilibration with all labile protons.

b) Assuming 4 labile protons.

fragment which is assumed to be largely the retro-Diels Alder product IV. The balance of the deuterium content must be in the 8 and 10-positions incorporation resulting from the initial



IV

addition of D^+ to the 8-position, and subsequent equilibration involving the tertiary carbonium ion.

Tritium labelled Δ^9-THC is prepared by a simple modification of the above procedure. The solution of Δ^8-THC in trifluoroacetic acid is saturated with lithium chloride and stirred for three days, when a quantitative yield of the tertiary chloride III is obtained. Methanolic hydrolysis of the 1-trifluoroacetate group, followed by treatment with potassium t-amylate in benzene according to the procedure of Petrzilka and Sikemeier (2), affords labelled Δ^9-THC.

We have made a cursory examination of the deuteration of other functionalities, employing a 10 mole % solution in CF_3COOD at room temperature, and following the deuterium incorporation by nmr and mass spectroscopic analysis. Representative examples are shown in Table 1, and it should be pointed out that the recorded deuterium incorporation can be easily increased by

either increasing the proportion of CF_3COOD, or repeating the equilibration. Cyclic and acyclic ketones (e.g., 2-methylcyclohexanone, 2-butanone) are rapidly labelled in the α-positions (half life <1 hr). Tertiary alcohols (e.g., triethylcarbinol) are converted to the trifluoroacetate and labelled (half life <2 hr) in the α-positions by an equilibrium process involving this ester and the corresponding olefin and carbonium ion. Secondary and primary alcohols (e.g., 2-propanol and 1-propanol) are not labelled at room temperature. The efficiency with which olefins may be labelled can be deduced from Peterson's extensive kinetic studies (3) of the addition of trifluoroacetic acid to a variety of alkenes and alkynes. In contrast to tetra- and trisubstituted olefins (cf. Δ^8-THC), addition of the acid to di- and mono-substituted olefins is slow at room temperature. For example, we find cyclohexene is converted to labelled cyclohexyl trifluoroacetate at room temperature in 2 days. Cyclohexene is converted to deuterated cyclohexyl iodide in >90% yield on treatment with lithium iodide in trifluoroacetic acid-d for 24 hr. Deuteration of aromatics only proceeds at room temperature when activating groups are present. For example, while p-cymene is not labelled at room temperature, anisole is selectively labelled in the ortho and para positions (half life ca. 48 hr), and both aromatic protons of the tetrahydrocannabinol system are rapidly exchanged.

Burnstein and Mechouloum (4) have reported that Δ^8-THC specifically labelled in the axial 10-position may be obtained by treating Δ^9-THC with p-toluenesulfonic acid-d in refluxing benzene. It is clear from our work that any synthesis along these lines must be carefully monitored if specific labelling is desired.

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