Synthesis of ^{3}H- and ^{14}C-labelled Tetrahydrocannabinols*

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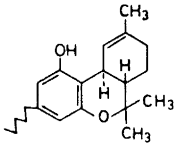
Cannabis, the resin of *Cannabis sativa* L., is at present the most widely illegally used drug. Surprisingly, so far only little pharmacological work has been carried out with pure cannabinoids and only limited information is available regarding the distribution, metabolism and elimination of these compounds.[1] This lack of information is undoubtedly partly due to difficulties in the preparation of these compounds in the radioactive labelled form, necessary to study the biotransformations of such active substances.

Δ^{1}-Tetrahydrocannabinol (Δ^{1}-THC) (*1*) $\Delta^{-1(6)}$-tetrahydrocannabinol (Δ^{6}-THC) (*5*) are considered to be the psychoactive constituents of *Cannabis sativa*.[1,2] In the present



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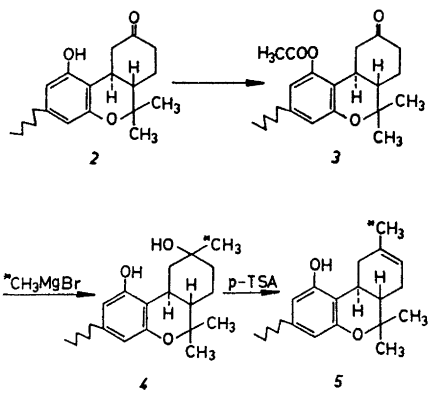
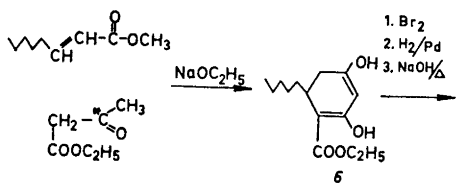
report we describe the first preparation of Δ^{1}-THC-^{3}H as well as the first two syntheses of specifically ^{14}C-labelled THC. We have prepared the ^{3}H-labelled Δ^{1}-THC by mild, acid catalyzed exchange of the aromatic protons in the molecule and ^{14}C-labelled Δ^{6}-THC by total synthesis of the molecule *via* two different routes as shown in Schemes 1 and 2. The radioactive Δ^{6}-THC can be converted to Δ^{1}-THC by the methods described by Mechoulam *et al.*[3] or by Petrzilka and Sikemeier.[4] It should be pointed out that the first route (Scheme 1), based on the work by Fahrenholtz *et al.*,[5] yields racemic Δ^{6}-THC while the second route (Scheme 2), which is



Scheme 1

based on the olivetol synthesis by Korte and Sieper [6] and the THC-synthesis by Petrzilka and Sikemeier,[7] produces (−)-Δ^{6}-THC.

^{14}C-Labelled Δ^{1}-THC has previously been obtained by Miras *et al.*[8] by growing Cannabis plants in $^{14}CO_2$-atmosphere, and



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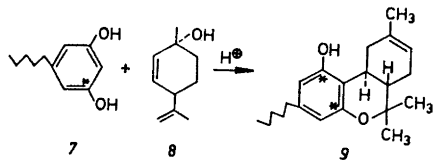

Scheme 2

^{3}H-labelled Δ^{6}-THC has been prepared by Burstein and Mechoulam [9] by acid catalyzed isomerization of Δ^{1}-THC to Δ^{6}-THC using tritiated water.

Experimental. Preparation of ^{3}H-labelled Δ^{1}-THC. Pure Δ^{1}-THC (47 mg) and phosphoric acid (100 mg; 100 %) were dissolved in tetrahydrofurane (0.5 ml). Tritiated water (0.05 ml;

* Part IV of Metabolism of Cannabis. Part III, Ref. 10.

5 C/ml) was added and the solution was heated to 80° for 2 h under nitrogen in a sealed ampoule. The reaction mixture was then dissolved in ether (25 ml) washed once with an equal volume of saturated $NaHCO_3$-solution and twice with water. After drying (Na_2SO_4) the ether was evaporated *in vacuo*. Residual exchangeable tritium was removed by dissolving the material in methanol (2 ml) and evaporating under a stream of nitrogen. This was repeated twice. GLC [10] (5 % SE-30 on Gas Chrom P, 225° and 5 % XE-60 on Chromosorb W AW-DMCS 225°) of the residue revealed that it consisted of 97 % Δ^1-THC. No peak due to Δ^6-THC appeared in the gas chromatogram. After purification by TLC on Silica gel G in ether/light petroleum (1:5) as solvent, 38 mg of pure (GLC) Δ^1-THC-^{3}H was obtained. The specific activies of several preparations ranged up to 140 μC/mg (44 mC/mmole). The tritium-label is sufficiently stable for biological work.[10]

Preparation of ^{14}C-labelled Δ^6-THC (Scheme 1). This synthesis was carried out by applying the route described by Fahrenholtz *et al.*[5] The ketone *2* (97 mg) [5] was dissolved in dry pyridine (1.5 ml) and acetic anhydride (1.5 ml) and the mixture was heated to 100° for 15 min. After cooling it was stirred with water (50 ml) for 30 min and then extracted with ether. The extract was washed with water (5 × 25 ml), dried (Na_2SO_4) and evaporated to give 130 mg of an oil identified as the acetylated ketone *3* by its IR-spectrum.

A Grignard reagent was prepared in ether (25 ml) using magnesium (200 mg) and methyl bromide (300 mg). After 30 min radioactive $^{14}CH_3Br$ (59 mg; 1 mC) was added and the mixture was stirred and refluxed for 30 min. A solution of *3* in ether (10 ml) was added and the reflux was continued for another 2 h. The mixture was then acidified with 2 N HCl, the layers were separated and the water layer extracted with ether. The ether extract was dried (Na_2SO_4) and evaporated to give *4* as a clear oil which was taken to the next step without purification.

Compound *4* was dissolved in benzene (100 ml), *p*-toluenesulphonic acid (10 mg) was added and the solution was refluxed for 4 h using a Dean-Stark trap. After cooling, the solution was washed with saturated $NaHCO_3$-solution and then with water, dried (Na_2SO_4) and evaporated. The residue was subjected to preparative TLC on Silica gel G using ether-light petroleum (3:10) as solvent and with Δ^6-THC as reference. The area corresponding to the reference was removed from the plate, eluted with ether which was then evaporated under a stream of nitrogen, affording 43 mg of an oil with a specific activity of 1.5 μC/mg. The compound (*5*) had identical chromatographic and spectral properties (GLC, TLC, IR) as an authentic sample of Δ^6-THC.

Preparation of olivetol-^{14}C (7) *and (−)-Δ^6-THC-^{14}C* (9) *(Scheme 2).* To sodium (88 mg) in absolute ethanol (2 ml) was added methyl 2-octanoate [6] (500 mg) and ethyl acetoacetate-3-^{14}C (500 mg; 0.5 mC) and the mixture was stirred and refluxed for 4 h. After cooling, the precipitate was separated by centrifugation, washed with absolute ethanol and dissolved in water (4 ml) at 0°. Concentrated HCl (0.8 ml) was added which precipitated an oil that was extracted into ether. The ether extract was dried (Na_2SO_4) and evaporated yielding 734 mg of the ester *6* as a pale yellow oil which was taken to the next step without further purification. The IR-spectrum of the preparation was in accord with that of an authentic sample.

The ester *6* (734 mg) and bromine (0.485 ml) were dissolved in acetic acid (2.5 ml) at 0° in an ampoule which was then sealed and heated to 60° for 4 h. After cooling, the mixture was diluted with water (3 ml) and the red oil that separated was dissolved in ethanol (50 ml) and triethylamine (5 ml) and hydrogenated over 10 % palladium on carbon (0.1 g) at 60 − 70° in a Parr apparatus (initial pressure 2.8 kg·cm^{-2}) over-night. The catalyst was removed by filtration through Celite and the solvent was evaporated *in vacuo*. The residue was dissolved in ether and subjected to preparative TLC on Silica gel G in ether/light petroleum (1:4) using authentic 6-carbethoxyolivetol as reference. The area corresponding to the reference was removed from the plates, eluted with ether and evaporation yielded 288 mg of 6-carbethoxyolivetol identified by its IR-spectrum and co-chromatography with an authentic sample. The specific activity was 0.35 μC/mg. The above ester (200 mg) was refluxed for 2 h in 2 ml 5 N aqueous NaOH. The solution was then acidified with HCl and refluxed for another 3 min, cooled and extracted with ether. The ether extract was dried (Na_2SO_4), evaporated and the residue was purified by preparative TLC in ether/light petroleum (1:5) and with authentic olivetol as reference. This afforded 94 mg ^{14}C-labelled olivetol (7) identified by its IR-spectrum and chromatographic behaviour. Specific activity 0.45 μC/mg.

The radioactive olivetol (90 mg), (+)-*trans*-*p*-menthadien-(2,8)-ol-(1) (96 mg) and *p*-toluenesulphonic acid (~5 mg) in dry benzene (10 ml) were reacted as described.[7] The solution was poured into 10 % aqueous $NaHCO_3$-solution (10 ml) and extracted with ether. After drying of the extract and evaporation of

the solvent, the residue was subjected to preparative TLC in ether/light petroleum (1:5) affording 103 mg of (−)-Δ^6-THC (*9*), ^{14}C-labelled in the aromatic ring. The compound had identical IR-spectrum, TLC- and GLC-behaviour as an authentic sample. Specific activity 0.31 μC/mg.

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The Basicities of Alkyl-1,3-dioxolanes and Their Implications to Hydrolytic Decomposition

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In previous studies [1,2] marked differences were found in the rates of hydrolysis of alkyl-1,3-dioxolanes and acyclic acetals. First, most of the acetone derivatives of 1,3-dioxolane hydrolyzed only one power of ten faster than the corresponding acetaldehyde derivatives although a rate increase by a factor of $10^{3.5}$ was expected due to the inductive polar effects. In the case of the fully methyl-substituted acetone derivative the rate was lower than that of the acetaldehyde derivative. Second, the rate differences between the isomeric compounds could not be explained to be consequences of initial state energy differences because the isomers of higher stability usually hydrolyzed more rapidly than the less stable forms. Third, the rate coefficients decreased with increasing number of 4- and 5-methyl substituents. These results were most reasonably explained by steric interactions.[1,2] As a consequence of the formation of a partial double bond in the attainment of the transition state of the hydrolysis, the bonds attached to the atoms of this double bond tend to become coplanar. Thus one of the substituents in position 2 must bend toward the average plane of the ring. It is obvious that this kind of change cannot take place without steric strain. Because steric effects are marked in the hydrolysis of most alkyl-1,3-dioxolanes, it is impossible to obtain information about other factors influencing the hydrolysis by comparing rate coefficients only. The basicities of various alkyl-1,3-dioxolanes were measured in this work to dissect the overall rates into partial factors, namely, the protonation pre-equilibrium constant and the rate coefficient of the rate-determining heterolysis.

Experimental. The samples of 1,3-dioxolanes used in the determinations of basicities were those that had been prepared for previous kinetic measurements.[1,2] The physical constants of the compounds were described in that