A NON-EXCHANGE SYNTHESIS OF TRITIUM-LABELLED TETRAHYDROCANNABINOLS.

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Received on October 5,1971.

SUMMARY

Tritium-labelled (-)-$\Delta^{1(6)}$ and (-)-Δ^{1}-tetrahydrocannabinols at a specific activity of 607 mCi/mmole were synthesized by a non-exchange method. The tritium was introduced by the catalytic reduction with tritium gas of an unsaturated precursor of dimethyl olivetol in hexafluorobenzene as a non-exchanging solvent. Specifically labelled olivetol was subsequently used in the synthesis of (-)-Δ^{1}-tetrahydrocannabinol by an established method. The radiochemical purity of the product was shown to be greater than 98% by TLC, GLC, liquid scintillation counting and bioassay.

The recent increased interest in the chemistry and pharmacology of the cannabinoids[1] has resulted in several methods[2]-[7] for the preparation of ^{3}H- and ^{14}C-labelled tetrahydrocannabinols (THC's). In the course of work on cannabis in this laboratory the synthetic sequence shown in Fig. 1 was used for the preparation of ^{3}H-(-)- Δ^{1}THC of high specific activity.

3',5'-Dimethoxyvalerophenone (1) was reduced with sodium borohydride to the alcohol (2) which was subsequently dehydrated to 1-(3,5-dimethoxyphenyl)pent-1-ene (3) with fused sodium bisulphate. The substituted styrene in hexafluorobenzene was hydrogenated with tritium gas to give 5-pentyl resorcinol dimethyl ether (dimethyl olivetol) which was specifically labelled in the side chain. Hexafluorobenzene was chosen as the solvent for the catalytic hydrogenation since it contained no protons

and tritium-exchange with solvent was thus impossible.

The tritium used in the reduction was diluted with hydrogen, the specific activity of the product being determined by the extent of this dilution. Theoretically, a maximum specific activity of 58 Ci/mmole is obtainable by this method but radiochemical decomposition would presumably impose a lower limit on the activity attainable.

The labelled dimethyl olivetol was demethylated with boron tribromide[8] in dichloromethane at room temperature and the resulting olivetol was condensed with (-)-verbenol (6) to give (-)-$\Delta^{1(6)}$THC.[9] After purification by column chromatography on Florisil, the labelled $\Delta^{1(6)}$THC was isomerised to Δ^{1}THC by the method of Petrzilka et al.[10].

After further column chromatography, the purity of the final product was demonstrated by TLC on silica gel; by its colour reaction with Fast Blue B; and by GLC on an XE 60 column[11] which resolved $\Delta^{1(6)}$THC and Δ^{1}THC. In these tests the behaviour of the labelled product was identical with that of an authentic sample of Δ^{1}THC. The radiochemical purity and specific activity were determined by chromatography on silica-gel-impregnated paper and liquid scintillation counting. The ^{3}H-Δ^{1}THC was obtained with a specific activity of 607 mCi/mmole and a radiochemical purity of greater than 98%.

A specific activity of 745 mCi/mmole had been expected and the observed radiochemical yield of 81% was ascribed to inadequate mixing of the tritium and hydrogen in the hydrogenation apparatus.

Using the mouse catalepsy bioassay procedure[12] at an intravenous dose level of 2 mg/kg, the product was equipotent with a sample of natural (-)-Δ^{1}THC isolated from Tincture of Cannabis B.P.C.[13].

The ^{3}H-Δ^{1}THC has been stored at a concentration of 250 µg/ml in benzene in brown bottles in the dark at 20° for twelve months without any marked chemical decomposition or loss of pharmacological activity.

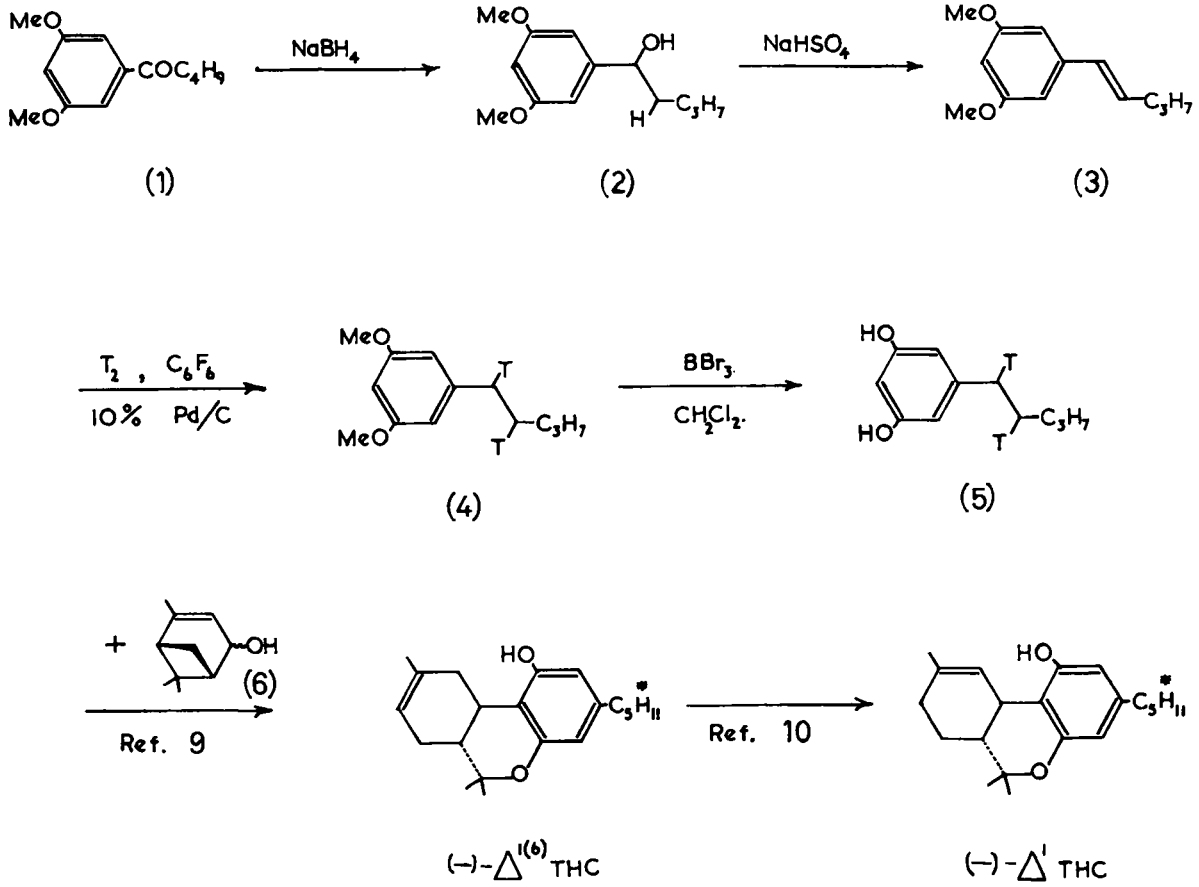
MeO
COC4H9
MeO
NaBH4
MeO
OH
MeO
H
C3H7
NaHSO4
MeO
MeO
C3H7
(1)
(2)
(3)
T2 , C6F6
10% Pd/C
MeO
T
MeO
T
C3H7
BBr3
CH2Cl2
HO
T
HO
T
C3H7
(4)
(5)
+
OH
(6)
Ref. 9
HO
C5H11
O
Ref. 10
HO
C5H11
O
(−)-Δ1(6) THC
(−)-Δ1 THC

Fig. 1

Contrary to the report of Suter and Weston,[14] we have found that 3,5-dimethoxyphenyl alkyl ketones are rapidly and completely hydrogenated in oxygenated solvents to give the corresponding 5-alkyl resorcinol dimethyl ethers[13] We attempted to use this reaction for the preparation of labelled dimethyl olivetol by the hydrogenation of 3',5'-dimethoxyvalerophenone with tritium gas over 10% palladium on charcoal in dried ethyl acetate. Under these conditions, however, exchange of tritium with solvent protons occurred to such an extent that the route was rendered impracticable. Hydrogenation of the ketone in non-oxygenated solvents such as benzene or carbon tetrachloride proceeded extremely slowly and was not useful for labelling purposes.

The method of choice for the preparation of specifically labelled ^{3}H-olivetol thus appears to be via the substituted styrene, but the catalytic hydrogenation of 3',5'-dimethoxyvalerophenone and subsequent demethylation with refluxing hydriodic acid or with boron tribromide provides the more convenient route for the bulk synthesis of unlabelled olivetol.

EXPERIMENTAL

Tritium gas was obtained from the Radiochemical Centre, Amersham.

The chloroform used as a solvent for TLC was B.D.H. reagent grade containing about 2% v/v ethanol as a preservative. Petroleum spirit b.p. 60-80° was redistilled before use. Dichloromethane was washed with concentrated sulphuric acid, water and 2N sodium carbonate, dried by refluxing with calcium hydride and distilled through a Vigreux column. TLC was carried out on Merck Kieselgel PF_{254}. GLC was carried out using a Pye Argon Gas Chromatograph; the column was 5% XE 60 on Supasorb (acid washed) 80-100 mesh (B.D.H.) at a temperature of 200° with an argon flow rate of 120 ml/minute. I.r. spectra were recorded on a Perkin Elmer 137B Infracord; n.m.r. spectra were recorded in carbon tetrachloride

at 100 MHz on a Perkin Elmer R14 spectrometer. Liquid scintillation counting was carried out on a Beckman LS-200B instrument using a dioxan-based scintillator containing Butyl PBD and naphthalene at concentrations of 7 and 50 g/l respectively. Solvents were removed in vacuo under nitrogen.

N,N-Diethyl-3,5-dimethoxybenzamide.

This compound was obtained from 3,5-dimethoxybenzoic acid via the acid chloride in the usual way. The amide was a very pale yellow solid m.p.78-79°, b.p. 158-160°/0.13 mmHg. ν_{max} in $CHCl_3$: 2980, 1640, 1610, 1465, 1425, 1160 cm^{-1}.

3',5'-Dimethoxyvalerophenone.

Sodium-dried ether (300 ml) was run into a 2 L flanged flask fitted with a stirrer, thermometer, gas inlet and pressure-equilibrating dropping funnel. The flask was flushed with nitrogen and all subsequent procedures up to the hydrolysis stage were carried out under a slight positive pressure of the gas. Freshly-cut lithium metal (8.7 g, 1.25 g atoms) was added in small pieces to the ether and the mixture was stirred. A crystal of iodine was added to the flask and 30 drops of a solution of n-butyl bromide (90 g, 0.65 moles) in dry ether (150 ml) were run in at room temperature to initiate the reaction. The rest of the solution of n-butyl bromide was then added dropwise with stirring at -10° (dry ice/trilene bath) over ½ hr. The mixture was then stirred at 0° (ice-bath) for 2 hrs. N,N-Diethyl-3,5-dimethoxybenzamide (118.5 g, 0.5 moles) in dry ether (150 ml) was added dropwise with stirring over 15 minutes at -25°. The mixture was stirred for 2 hrs at -10° and for 5 hrs at 0°. The mixture was then allowed to reach room temperature and stirring was continued over a further total period of 11 hrs. The mixture was cooled to -25° and saturated ammonium chloride solution (350 ml) was added cautiously, maintaining the temperature at below -20°. After completion of the addition, water (100 ml) and ether (250 ml) were

added with vigorous stirring; the contents of the flask were then allowed to reach room temperature and transferred to a separating funnel. After further dilution with water (250 ml), the layers were separated and the aqueous layer was re-extracted with ether (2 x 100 ml). The combined extracts were washed with 2N hydrochloric acid, distilled water and saturated brine (250 ml of each); dried ($MgSO_4$), and filtered and the ether was evaporated in vacuo. The residual yellow oil was distilled in vacuo. No clear fractionation between the ketone and unreacted amide was observed, but the main fraction (82.7 g) of the distillate boiling between 140-170° at 0.13-0.20 mmHg consisted mainly of Ketone. This fraction was a pale yellow oil which crystallised to a waxy solid on standing. It was recrystallised from ethanol/water at 50° with rapid cooling in dry ice/trilene. Yield 67.0 g (60.4%). After a further recrystallisation, the product melted at 38-39° (Lit.[14] 42.5°). Further quantities of the ketone of lower purity were obtainable by the distillation in vacuo of the final fraction (22.8 g) from the first distillation and from the mother liquors from the recrystallisation. ν_{max} in CCl_4: 2950, 1705, 1610, 1465, 1430, 1355, 1298, 1212, 1160, 1070, 1032 cm^{-1}.

1-(3,5-Dimethoxyphenyl)pentan-1-ol.

To 3', 5'-Dimethoxyvalerophenone (22.2 g, 0.1 mole) in methanol (100 ml) was added sodium borohydride (2 g, 0.053 moles) in methanol (50 ml) made alkaline with a few drops of caustic soda. The mixture was refluxed for 2 hrs on a water-bath, cooled, poured into 2N hydrochloric acid (100 ml) diluted with water (200 ml) and extracted with ethyl acetate (2 x 100 ml). The combined extracts were washed with 1N sodium bicarbonate solution (50 ml) and saturated brine (50 ml), dried ($MgSO_4$), filtered and the solvent was evaporated in vacuo. The residue was distilled in vacuo to give a colourless, fairly viscous oil (20.0g, 89.2%), b.p. 130-148° at 0.08 mmHg; $n_D^{23.5}$ = 1.5192; Found: C 69.9%, H 8.8%, $C_{13}H_{20}O_3$ requires

C 69.61%, H 8.99%. ν_{Max} in $CHCl_3$: 3400 (weak, br, OH), 2900, 1725, 1600, 1455, 1420, 1290, 1190, 1150, 1057, 922 cm^{-1}. τ 9.17 (t, J = 6Hz, terminal methyl), 8.3 - 9.0 (aliphatic $-CH_2-$), 6.33 (s, CH_3O-), 5.64 (t, J = 8 Hz, $-\overset{*}{C}H(OH)-$), 3.84 (d, J=2 Hz, single aromatic proton), 3.71 (d, J = 2Hz, two aromatic protons).

1-(3,5-Dimethoxyphenyl)pent-1-ene.

In the boiling flask of a semi-micro vacuum distillation apparatus equipped with a dropping funnel and nitrogen bleed were placed powdered potassium bisulphate (2 g) and a few small crystals of p-tert-butyl catechol. The flask was filled with nitrogen, heated to 235° on an oil-bath and evacuated to 15 mmHg. 1-(3,5-Dimethoxyphenyl)pentan-1-ol (5 g) was added dropwise over 10 minutes and the crude product was collected as a clear distillate (1.5 g). It was dissolved in sodium-dried ether and dried with $MgSO_4$. After filtration, and evaporation of solvent, the product was purified by column chromatography on Florisil (40 g), eluting with 1% v/v ether in petroleum spirit (b.p. 60-80°). 1-(3,5-dimethoxyphenyl) pent-1-ene was obtained as a colourless, mobile oil, n_D^{20} = 1.5430 (1.3 g, 28.3%). ν_{max} in CCl_4: no hydroxyl, 3950, 1600, 1465, 1425, 1210, 1155, 1067, 965 cm^{-1}. Found C 76.2%, H 8.8%. $C_{13}H_{18}O_2$ requires C 75.69, H 8.80%. τ 9.04 (t, J = 7 Hz, terminal methyl), 8.51 (sextet, J = 7 Hz, methylene adjacent to methyl), 7.84 (quartet, J = 6 Hz, methylene adjacent to vinylic proton), 6.26 (s, CH_3O-), 3.88 (d, J = 6 Hz, vinylic proton adjacent to methylene), 3.80 (d, J = 6 Hz, vinylic proton adjacent to ring), 3.78(d, J = 2 Hz, single aromatic proton), 3.63 (d, J = 2 Hz, two aromatic protons).

^{3}H-Dimethyl Olivetol

1-(3,5-Dimethoxyphenyl)pent -1-ene (1.034 g, 5 mmoles) in hexafluro-benzene (4 ml) was hydrogenated with magnetic stirring over 10% palladium on charcoal (100 mg) using a mixture of tritium (5 Ci, in a break-seal ampoule) and hydrogen (150 ml) in a gas burette containing dibutyl phthalate.

Hydrogen uptake was rapid and 137 ml (22% excess) was absorbed in 15 minutes, when the reaction vessel was isolated from the hydrogenation apparatus. Excess tritium was removed by flushing with hydrogen (2 x 100 ml) and catalytically reducing cyclohexene contained in a second reaction vessel. The solution of the hydrogenated product was centrifuged to remove the catalyst and the supernatant was evaporated in vacuo at 40°. The residue was taken up in redistilled methanol (3 x 25 ml) with intermediate evaporation in vacuo at 40° to remove any traces of exchangeable tritium and it was then dissolved in purified dichloromethane (25 ml) and cooled to 0°.

^{3}H-Olivetol.

To the cooled solution of ^{3}H-dimethyl olivetol in dichloromethane was added boron tribromide (2 ml) in dichloromethane (25 ml) at 0°. The reaction vessel was quickly fitted with a $CaCl_2$ tube and was allowed to stand at room temperature for 3½ hr. The contents were then pipetted into a mixture of sodium sulphite (1 g), water (50 ml) and crushed ice (50 g) in a 500 ml separating funnel. Ethyl acetate (50 ml) was added and after shaking, the lower organic layer was separated. The aqueous layer was re-extracted with ethyl acetate (50 ml, upper layer). The combined extracts were washed with 1N sodium bicarbonate (2 x 20 ml), and saturated brine (2 x 25 ml) and the solvent was evaporated in vacuo at 40°. The reddish oily residue was heated in vacuo at 80° for ¼ hr to decompose any brominated products and was then redissolved in ethyl acetate (25 ml). The solution was washed with 5% w/v sodium sulphite solution (1 x 10 ml) 1N sodium bicarbonate (1 x 10 ml), and saturated brine (2 x 10 ml) and then dried ($MgSO_4$). After filtration, and evaporation of solvent in vacuo at 40° the residue was taken up in ethyl acetate (3 ml), applied to a column of Florisil (40 g) and rapidly eluted with ethyl acetate to separate olivetol from immobile by-products. The first 150 ml of eluate were collected and evaporated in vacuo at 40° to yield an almost colourless oil which was immediately redissolved in

dichloromethane (100 ml) and stored in the dark at -10°. On TLC the product appeared as a single red spot R_F = 0.60, with slight upward streaming (silica gel; ethyl acetate; reagent: anisaldehyde (0.5 ml) in glacial acetic acid (10 ml), methanol (85 ml) and concentrated sulphuric acid (5 ml); spray and heat at 100° for 10 minutes.)

^{3}H-(-) $\Delta^{1(6)}$THC

To the ^{3}H-olivetol in dichloromethane (100 ml) were added a mixture of cis- and trans-(-) verbenols[15] (1 g) in dichloromethane (50 ml) and boron trifluoride etherate (1 ml) in dichloromethane (50 ml). The stoppered mixture was allowed to stand for 3 hrs at room temperature and was then washed with 1N sodium bicarbonate (2 x 50 ml), distilled water (1 x 25 ml), and saturated brine (2 x 25 ml). After drying ($MgSO_4$), filtration, and evaporation of solvent *in vacuo*, the product was purified by column chromatography on Florisil (330 g), eluting with 2% v/v ether in petroleum spirit (b.p. 60-80°) Fractions of eluate were monitored for cannabinoids by spotting on filter paper and spraying with 0.1% w/v aqueous Fast Blue B. The fractions which gave a positive reaction were analysed by TLC (silica gel, 2% v/v methanol in chloroform) using an authentic sample of $\Delta^{1(6)}$THC as a marker. The first, minor, component off the column had R_F = 0.70 and gave a red-brown spot with Fast Blue B. ^{3}H-$\Delta^{1(6)}$THC was the second component, R_F = 0.60 and gave a red-purple colour reaction. After evaporation of solvent *in vacuo*, the residual oil was redissolved in dry benzene (100 ml) and stored in the dark under nitrogen at room temperature.

^{3}H-(-)- Δ^1THC

After evaporation of the benzene *in vacuo* at 40°, the ^{3}H- $\Delta^{1(6)}$THC was dissolved in dichloromethane (10 ml) and zinc chloride (150 mg) was added. Hydrogen chloride was bubbled into the mixture at 0°, with

occasional shaking, for 20 minutes, a further 5 ml of dichloromethane being added after 10 minutes to replace losses by evaporation. The flask was then tightly stoppered and the mixture was stirred magnetically for 20 hours. The yellow-brown solution was diluted with ether (100 ml), shaken with ice-water (20 ml) and washed with 1N sodium bicarbonate (2 x 20 ml) and saturated brine (2 x 10 ml). After drying ($MgSO_4$), filtration, and evaporation of solvent in vacuo, the residual ^{3}H-1-chlorohexahydrocannabinol was taken up in sodium-dried benzene (5 ml). Potassium metal (250 mg) was dissolved, with warming, in t-amyl alcohol (8 ml) which had been dried by refluxing with calcium hydride. The excess alcohol was evaporated in vacuo at 50° and the residual potassium t-amylate was dissolved in dry benzene (5 ml) and cooled to 0°. The apparatus was flushed with argon and the solution of ^{3}H-1-chlorohexahydrocannabinol in benzene was added dropwise over 12 minutes. The mixture was then warmed at 65° with agitation by a stream of argon for 15 minutes. Carbon dioxide was then passed through the mixture at 0° for 30 minutes. The resulting yellow solution was diluted with benzene (90 ml) and washed with 1N sodium bicarbonate (2 x 20 ml), distilled water (1 x 20 ml), and saturated brine (2 x 20 ml). After drying ($MgSO_4$), filtration, and evaporation of solvent in vacuo at 40°, the residue was chromatographed on Florisil (80 g), with 2% v/v ether in petroleum spirit (b.p. 60-80°) as eluent. Fractions were again monitored by spotting on filter paper and by TLC. ^{3}H-Δ^1THC was the first component off the column and was obtained as a purplish oil (230 mg, 14.6% based on 1-(3,5-dimethoxyphenyl) pent-1-ene), which gave a red-purple spot, R_F = 0.60, with East Blue B. The gas chromatogram of the product showed that it consisted of more than 98% Δ^1THC (retention time 17.5 minutes), the only detectable impurity being $\Delta^{1(6)}$THC (retention time 14.75 minutes). The product was stored at room temperature in brown bottles in the dark in 1 l of dry benzene.

Radiochemistry

The radiochemical purity was demonstrated by TLC analysis of the product on silica gel with ether as solvent (R_F of Δ^1THC ca. 0.73). 1.5 cm strips of gel were scraped into tubes and extracted with dioxan. After centrifugation, aliquots of supernatant were counted by liquid scintillation counting. The product was also chromatographed on Whatman SG 81 silica-gel impregnated paper using 1% v/v methanol in chloroform as solvent (R_F of Δ^1THC ca. 0.65). The chromatogram was cut into 1 cm wide strips which were then extracted by the scintillator solution directly in the counting vials. Both methods showed that more than 98% of the radio-activity was concentrated at the R_F value of authentic Δ^1THC. The specific radioactivity of the product was obtained by dilution of the stock solution and liquid scintillation counting. It was found to be 607 mCi/mmoles (1.9 mCi/mg).

ACKNOWLEDGMENT

This work was supported by the Medical Research Council. Gareth Jones is grateful to the M.R.C. for the award of a scholarship for Training in Research Methods.

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