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Synthesis of a potential urinary tetrahydrocannabinol metabolite

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SUMMARY 7-Hydroxy-$\Delta^{1,6}$-tetrahydrocannabinol was methylated on the phenolic hydroxyl groups whereupon the hydroxymethyl group was oxidized, using chromic acid or MnO_2, to the corresponding crystalline aldehyde 7. This was further oxidized to the corresponding carboxylic acid methyl ester *8* using MnO_2 and NaCN in methanol. When the ester *8* was compared chromatographically with the methylated acidic metabolites of Δ^1-tetrahydrocannabinol, the metabolite(s) turned out to be more polar than the synthetic compound, indicating that the metabolite(s) appear to have more polar groups than the synthetic molecule.

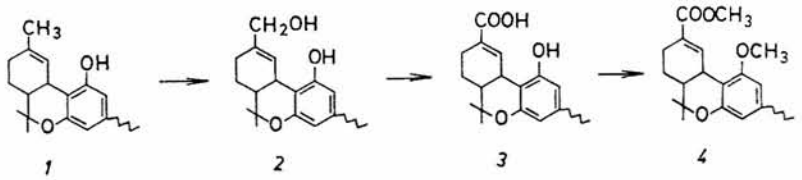
[1] Metabolism of Cannabis XII, Part XI see ref. [14].

It has recently been demonstrated that tetrahydrocannabinol [1—4] (Δ^1- and $\Delta^{1,6}$-THC; *1* and *5*), cannabinol [5—6] and cannabidiol [7] are all primarily converted to hydroxymetabolites by the liver microsomal fraction. However, these products are apparently not eliminated unchanged, but further converted into acidic metabolites [8] which are excreted as such or as conjugates [9]. The acidic metabolites of THC in the urine of rabbits have now been further investigated.

Isolation and synthesis of the potential metabolite

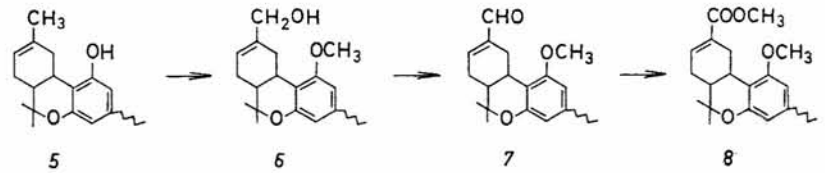
^{14}C-Labelled Δ^1-THC was administered intravenously to rabbits and the urine was collected for 24 hours. Acidic metabolites were separated from the urine and purified by extraction and chromatographic methods as discribed in the experimental section.

Based on the present knowledge of THC-metabolism, it is conceivable that an acidic metabolite of Δ^1-THC is formed by an initial hydroxylation to 7-hydroxy-Δ^1-THC (*2*) and subsequent oxidation of the hydroxymethyl group to a carboxylic acid of structure *3* (Scheme 1)



Scheme 1

Since only microgram quantities of the acidic metabolites were obtained, we considered combined gas chromatography-mass spectroscopy to be the most useful tool for the identification of the isolated metabolite(s). The isolated material was therefore methylated on the assumption that it would yield the anticipated structure *4*. The closely related compound *8* (with the double bond in the $\Delta^{1,6}$-position) was synthesized for reference purposes as shown in Scheme 2.



Scheme 2

In this synthesis, 7-hydroxy-$\Delta^{1,6}$-THC was methylated on the phenolic hydroxyl group to *6* and this was then oxidized using either chromic acid in methylene chloride or manganese dioxide in hexane to give the crystalline aldehyde *7* in good yield. The aldehyde was further oxidized using MnO_2 in methanol to form the methyl ether *8* in 60 % yield. Oxidation of the aldehyde using silver nitrate was less successful.

The synthetic ester *8* was compared with the methylated acidic isolated metabolite(s) of Δ^1-THC using TLC. However, the isolated material turned out to be more polar than the synthetic compound, and consequently the acidic metabolite(s) can be assumed to have more polar groups than the synthetic molecule. Two such polar acidic metabolites have recently been reported by Burstein *et al.* [9]. These were directly compared with our synthetic material by Dr. Burstein and were also found to be more polar.

The synthetic route to acidic metabolites from THC outlined in this paper should equally well apply to metabolites containing hydroxyl groups apart from the carboxylic acid function. We conclude that if the acid *3* is a metabolite of Δ^1-THC in the rabbit, it is formed in only minute quantities.

Experimental

General comments

IR spectra were measured on a Perkin-Elmer 237 spectro-photometer and UV spectra were recorded on a Beckman DK-2A photometer. NMR spectra were obtained using a Varian A 60 instrument and mass spectra were recorded on an LKB 9000 apparatus at 70 eV. Thin-layer chromatography was performed as previously described [11].

Isolation procedure

Two female albino rabbits (2 kg) were injected i.v. with a propylene glycol solution of ^{14}C-Δ^1THC [10] (2 mg/kg; 75.000 dpm/mg THC) and the urine was collected during 24 hours. After adjustment of the pH to 7, the urine was applied to a column of Amberlite XAD-2 [12] (500 ml of urine to 200 g of resin). The column was washed with water to remove inorganic salts, and the metabolites were eluted with methanol. The eluate was evaporated to dryness and partitioned between a buffer (pH = 3) and ether. By this method, 70 % of the eluted radioactivity was recovered in the organic layer. The ether solution was shaken with 5 % aqueous $NaHCO_3$ which extracted all the radioactivity into the water layer, and after acidification to pH 3, the metabolites were reextracted into ether, and the extract evaporated to dryness.

Methylation of the metabolite mixture

The extracted material (2 % metabolites) was dissolved in methanol (1 ml) and refluxed for 10 min under nitrogen with dimethyl sulphate (0.5 ml) and aqueous sodium hydroxide (0.05 ml; 5 *N*) to methylate the phenolic hydroxyl group(s). The solution was then acidified to pH 3, extracted with ether, the extract dried (Na_2SO_4) and evaporated to dryness. This material was further methylated with diazomethane in ether.

TLC of the reaction products on Silica Gel G plates with ether as solvent gave radioactive compounds with roughly the following R_f-values: Non-methylated $R_f = 0.0$; methylation with dimethylsulphate $R_f = 0.2$; methylation with dimethyl sulphate and diazomethane $R_f = 0.9$. The material thus obtained was a mixture of several radioactive components as indicated by TLC; together approximately 100 μg of metabolites.

Synthetic procedures

7-Hydroxy-$\Delta^{1,6}$-THC methyl ether (6). A mixture of 7-hydroxy-$\Delta^{1,6}$-THC [13, 14] (722 mg; 2.2 mmole), methyl iodide (315 mg; 2.2 mmole) and K_2CO_3 (3 g) in DMF (30 ml) was left at room temperature over night. The mixture was the poured into water (30 ml) and extracted three times with ether. The combined etheral fractions were washed with water and dried over $MgSO_4$. After evaporation, the obtained material (757 mg), as purified by chromatography on Silica Gel (35 g; 0.05—2 mm, Merck). Elution with 20 % ether in light petroleum (b.p. 40—60°C) gave 715 mg (96 %) of *6*, pure by GLC (2 % OV-17 on Gas Chrom Q, N_2 flow rate 30 ml/min, column temp. 235°C, glass column 6 ft×1/8 inch). $[\alpha]_D^{20} = -246°C$ (ethanol).

Anal. ($C_{22}H_{32}O_3$): C and H. ν_{max} (CCl_4) 3600 cm^{-1} (OH). λ_{max} (hexane) 215 nm ($\varepsilon = 34400$), 225 nm (sh.) $\varepsilon = 23600$, 271 nm ($\varepsilon = 1158$) and 274 nm ($\varepsilon = 1226$). NMR: 6.18 and 6.12 ppm (s, 1H each, Ar*H*), 5.60 ppm (m, 1H, vinylic), 4.90 ppm (s, 2H, $-CH_2OH$ and 4.45 ppm (s, 3H, $-OCH_3$).

MS: Prominent peaks at m/e (rel. int. %): 344 (16) M^+. 326 (7), 313 (6), 311 (8), 288 (15), 260 (15), 245 (100), 244 (18), 207 (44), 188 (21), 174 (19), 165 (16), 145 (18), 138 (25), 132 (22), 119 (22), 115 (26), 105 (22), 91 (42), 81 (21), 79 (30), 77 (22) and 55 (32).

Oxidation of the alcohol 6 to the aldehyde 7

1. Chromic acid oxidation (based on the work by Collins *et al.* [15]).

To a solution of *6* (510 mg; 1.4 mmole) in dry methylene chloride (10 ml) was added chromic acid/pyridine complex (1.5 g; 8.4 mmole) and the mixture was stirred for 1/2 hour and then poured into water and extracted three times with ether. The combined extracts were washed with 10 % aq. HCl and with water and then dried ($MgSO_4$). On evaporation, 506 mg material was obtained, which was chromatographed on Silica Gel (25 g). The material eluted with 20 % ether in light petroleum was crystallized from ether/light petroleum to give 304 mg (59 %) of *7* m.p. 137°C $[\alpha]_D^{20} = -354°C$ (ethanol).

Anal. ($C_{22}H_{30}O_2$): C and H. ν_{max} (KBr): 1710 cm^{-1} (−CHO) λ_{max} (hexane): 212 nm ($\varepsilon = 40700$), 226 nm ($\varepsilon = 29300$), 272 nm ($\varepsilon = 1140$), 279 nm ($\varepsilon = 1209$). NMR: 9.60 ppm (s, 1H, $-CHO$), 6.9 ppm (m, 1H, vinylic), 6.40 ppm (d, 2H, $J \sim 2$ Hz., Ar*H*) and 3.85 ppm (s, 3H, $-OCH_3$).

MS: Prominent peaks at m/e (rel. int. %): 342 (8) M^+. 326 (4), 313 (4), 311 (4), 286 (28), 271 (11), 260 (16), 259 (11), 246 (18), 245 (100), 201 (14), 188 (15), 138 (20), 128 (13), 119 (16), 115 (16), 107 (14), 105 (15), 91 (30), 79 (16) and 77 (18).

2. *Manganese dioxide oxidation* (based on the work by Corey *et al.* [16]).

To a suspension of manganese dioxide (119 mg; 1.36 mmole) and sodium cyanide (17 mg; 0.35 mmole) in hexane (20 ml) and approximately 10 mg of acetic acid was added 7-hydroxy-$\Delta^{1,6}$-THC methyl ether (*6*) (23 mg; 0.067 mmole) at 0°C and the mixture was stirred at that temperature for 3 hours. It was left at 0°C over night, filtered and evaporated. The residue (23 mg) crystallized and was recrystallized once from light petroleum, m.p. 132—134°C, to yield 18 mg (79 %) of *7*, having the same spectral data as presented above.

Oxidation of the aldehyde 7 to the ester 8 (based on the work by Corey *et al.* [16])

The aldehyde *7* (23 mg; 0.067 mmole) in dry methanol (5 ml) was stirred overnight at room temperature with manganese dioxide (119 mg; 1.36 mmole), sodium cyanide (17 mg; 0.35 mmole) and approximately 10 mg of acetic acid. After filtration and evaporation, the residue was partitioned between water and ether, the ether layer dried (Na_2SO_4) and evaporated. The residue (23 mg) was purified by preparative TLC in ether/light petroleum (1:1) to yield 15 mg (60 %) of the ester *8* as an oil.

Anal. ν_{max} (film) 1700 cm^{-1} (C=O). NMR: 7.1 ppm (m, 1H, vinylic), 6.38 ppm (d, 2H, J ~ 2 Hz. Ar-*H*), 3.85 and 3.78 ppm (s, 3H each, $-OCH_3$ and $-COOCH_3$). MS: Prominent peaks at m/e (rel. int. %): 373 (18) M + 1, 372 (73) M^+. 357 (7), 341 (13), 330 (12), 317 (15), 316 (67), 297 (32), 260 (19), 245 (100), 207 (33), 190 (10), 178 (11), 143 (9), 137 (12), 119 (17), 105 (8) and 91 (13).

Oxidation of the aldehyde *7* using $AgNO_3$ according to Borowiecki and Reca [17] gave the ester *8* in about 6 % yield.

Chromatographic comparison between isolated material and the ester (8)

The dimethylated radioactive isolated material described above was chromatographed on 20×20 cm Silica Gel G plates in ether/light petroleum (1:1) and with the synthetic ester *8* as reference. The reference spot was detected by spraying the plate with $KMnO_4$-solution, and the methylated metabolites by determining the radioactivity on the plate by counting the scraped-off adsorbent.

The reference compound appeared at $R_f = 0.8$ and the least polar part of the radioactivity (50 %) was concentrated in a 1 cm broad band at $R_f \sim 0.4$. The remaining radioactivity was more polar.

Acknowledgements

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