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A Urinary Metabolite of Δ^1-Tetrahydrocannabinol. The First Synthesis of 4"-Hydroxy-Δ^1-Tetrahydrocannabinol-7-oic Acid Labelled with Deuterium

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SUMMARY

The first synthesis of 4"-hydroxy-Δ^1-THC-7-oic acid, one of the three major metabolites of Δ^1-THC identified in human urine is discussed. Methyl 4-(3,5-dihydroxyphenyl)butanoate (**8**) was prepared from 3,5-dihydroxybenzoic acid in an overall yield of 15%. **8** was condensed with a terpene synthon (**9**) under acidic conditions followed by hydrolysis and conversion of the 4"-carboxylic acid function to the corresponding methyl ketone using methyllithium. Reduction with $NaBH_4$ afforded the secondary alcohol in the side-chain. Acetylation and removal of the 1,3-dithiane masking group gave the aldehyde in C-7-position which was further oxidized using $NaClO_2$ followed by deacetylation to give the desired metabolite **14**. The same procedure may be used for the synthesis of unlabelled 4"-hydroxy-Δ^1-THC-7-oic acid.

Key Words: Deuterated 4"-hydroxy-Δ^1-THC-7-oic acid; Urinary metabolite; Synthesis; Cannabis

INTRODUCTION

The metabolism and pharmacokinetics of Δ^1-tetrahydrocannabinol (**1**, Δ^1-THC), the major psychoactive component of *Cannabis sativa* L., have been studied in several species including man [1]. Differences in the complex metabolic pattern seem to be quantitative rather than qualitative. To date about 100 metabolites have been identified from *in vivo* and *in vitro* studies. The major metabolic route is hydroxylation at the allylic 7-position followed by oxidation of the resulting alcohol to the corresponding carboxylic acid. Abundant metabolites are also formed by oxidative processes in the allylic 6α- and/or 6β-positions as well as in the side-chain leaving the length of the pentyl carbon

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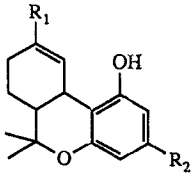
chain either intact or shortened with an additional carboxylic acid function. In man, the three major urinary metabolites identified so far are Δ^1-THC-7-oic acid (**2**), 4",5"-bisnor-Δ^1-THC-7,3"-dioic acid (**3**) and 4"-hydroxy-Δ^1-THC-7-oic acid (**4**), accounting for about 40% of the amount of metabolites excreted in the urine after oral administration [2].



1 $R_1 = CH_3$ $R_2 = C_5H_{11}$
2 $R_1 = COOH$ $R_2 = C_5H_{11}$
3 $R_1 = COOH$ $R_2 = C_2H_4COOH$
4 $R_1 = COOH$ $R_2 = C_3H_6CH(OH)CH_3$

Fig 1: Structures of Δ^1-THC (**1**) and three major human urinary metabolites (**2-4**).

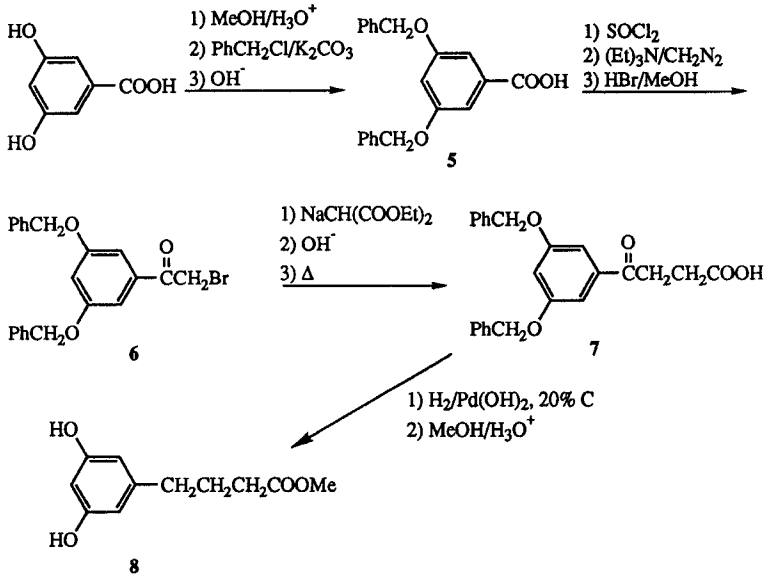
Abuse of marijuana or other products of the cannabis plant can be detected through the analysis of various biological fluids, usually plasma and urine, for the parent compound, Δ^1-THC or its major metabolites. The urinary metabolites are mainly eliminated as conjugates, most likely glucuronides, but have so far only been detected as unconjugated acids after hydrolysis. Determination of abuse is of interest not only in forensic science, but also as a means of ensuring that employees in vital positions, e.g. pilots, surgeons or professional drivers, are not habitual users of marijuana, which could present a danger to others.

Immunoassays, such as EMIT and RIA, are often used to detect the presence of the major urinary metabolite, Δ^1-THC-7-oic acid. However, the method of choice for verification and quantitation of the parent compound, Δ^1-THC, and Δ^1-THC-7-oic acid at nano or picogram levels is gas chromatography-mass spectrometry (GC/MS) [3]. Measurements can be difficult due to the low levels encountered and the presence of co-extracted endogenous compounds possessing similar chromatographic properties. In the analysis of **2** using GC/MS in the Selected Ion Monitoring mode (SIM), interference by a minor THC metabolite, 6-hydroxy-3",4",5"-trisnor-Δ^1-THC-2"-oic acid and by urinary metabolites of drugs as well as endogenous urinary components has been reported [4-6]. For more accurate detection of cannabinoids in biological fluids, the synthesis of metabolites other than Δ^1-THC-7-oic acid is needed for use as additional references and internal standards. The internal standards most suitable for mass spectral analysis are deuterated analogues of the drug to be

analyzed, because they demonstrate essentially the same partitioning, chromatographic and derivatizing behaviour [7]. Several reports on the synthesis of Δ^1-THC-7-oic acid can be found in the literature and the synthesis of Δ^1-THC-7-oic acid in unlabelled and deuterium labelled form has previously been presented [8-11]. Recently, the first and only synthesis of unlabelled and deuterium labelled 4",5"-bisnor-Δ^1-THC-7,3"-dioic acid, the major dicarboxylic acid metabolite in man, was reported [12]. 4"-Hydroxy-Δ^1-THC-7-oic acid (**4**) is the third most abundant urinary metabolite of Δ^1-THC identified so far in man [2]. To our knowledge, the synthesis of this metabolite has not been reported before. In this paper, the first synthesis of (±)-4"-hydroxy-Δ^1-THC-7-oic acid (**14**) as its deuterated analogue is presented in detail.

MATERIAL AND METHODS

3,5-Dihydroxybenzoic acid was purchased from Aldrich-Chemie, Germany. Melting points were determined on a Electrothermal Digital melting point apparatus and are uncorrected. Infrared spectra were recorded on a Polaris FT-IR spectrometer (Mattson Instruments, UK) using KBr pellets, a neat film between salt plates or a $CHCl_3$ solution. ^1H NMR and ^{13}C NMR spectra were recorded on a JEOL JNM-EX 270 MHz instrument a Varian Gemini 300 or a Varian Unity 300 spectrometer. Spectra were recorded with TMS as the internal standard in [^2H]-chloroform, unless otherwise stated. Two-dimensional NMR spectroscopy was performed with standard C-H heteronuclear correlation. Attached Proton Test (APT) [13] or DEPT was used to determine the carbon multiplicity. The isotopic distribution was estimated by mass spectrometric analyses using a Finnigan MAT TSQ 70 (San José, CA, USA) equipped with a Finnigan MAT thermospray source (ammonium acetate was used as the ionizing agent) or by using GC/MS equipped with an HP Ultra 2 column (5% phenylmethyl silicone; 25 m x 0.32 mm x 0.52 μm) connected to a Finnigan MAT SSQ 700 (San José, CA, USA). Before GC/MS analysis the compounds were esterified with CH_2N_2 and derivatized by silylation with N,O-bis(trimethylsilyl)-acetamide in anhydrous MeCN. Silica gel 60 (70-230 mesh, Merck) was used for open bed liquid chromatography. Low pressure liquid chromatography was performed using a pre-packed normal phase (Si 60, 40-63 μm) Lobar column (Merck). The monoterpenoid numbering system is used throughout this paper. For numbering of the atoms in molecules **10-14** see scheme 2.



Scheme 1: Preparation of methyl 4-(3,5-dihydroxyphenyl)butanoate (8).

EXPERIMENTAL

3,5-Dibenzyloxybenzoic acid (5; See scheme 1)

Benzyl chloride (21 ml, 182.6 mmol) and K_2CO_3 (10.75 g, 77.9 mmol) were added to a solution of methyl 3,5-dihydroxybenzoate (12.40 g, 73.8 mmol), prepared by standard methods, in absolute acetone (60 ml) while stirring. The reaction mixture was refluxed for 20 hrs, diluted with toluene (300 ml), and washed with H_2O (3 x 300 ml). The combined organic layers were dried over Na_2SO_4 and evaporated to give a crude mixture in 70% yield which crystallized upon standing. The crystals of methyl 3,5-dibenzyloxyphenylbenzoate were washed with petroleum ether. Mp and spectral data were in agreement with previously published data [14].

Methyl 3,5-dibenzyloxyphenylbenzoate (7.68 g, 22.1 mmol) was dissolved in acetone (100 ml) and 10% aq. NaOH (23.6 ml) and refluxed for 2 hrs. The reaction mixture was concentrated, poured onto ice water (100 ml), and acidified with 2 N HCl to pH 3-4. The crystals of **5** (yield 90%) were washed with EtOAc and thoroughly dried before being used in the subsequent reaction. Mp and spectral data were in agreement with previously published data [15].

3,5-Dibenzyloxyphenacyl bromide (6)

A solution of **5** (14.00 g, 41.9 mmol) and $SOCl_2$ (38.5 ml, 52.8 mmol) in anhydrous benzene (40 ml) was refluxed for 5 hrs. Removal of excess $SOCl_2$ and benzene from the reaction mixture by distillation at reduced pressure afforded brownish crystals. Recrystallization from petroleum ether resulted in white needles in 65% yield (9.60 g, 27.2 mmol). Mp 74.5-75.2°C (lit. 73-74°C) [16]. ^{1}H NMR δ 7.38 (m, 12 H, Ph; H-2; H-6), 6.88 (t, J = 2.3 Hz, 1 H, H-4), 5.05 (s, 4 H, PhCH_2O).

A solution of $(Et)_3N$ (6 ml, 43.1 mmol) in CH_2Cl_2 (80 ml) was mixed with a slight excess of CH_2N_2, freshly prepared by well established procedures, under cooling to -15°C with dry ice and EtOH. The corresponding acid chloride of **5** (6.97 g, 19.8 mmol) was added in small portions while stirring. The reaction mixture was kept at 0°C for 1-2 hrs and then at room temperature over night, evaporated to dryness under reduced pressure and then dissolved in CH_2Cl_2 (120 ml). The organic layer was washed with H_2O (3 x 100 ml), dried over Na_2SO_4 and evaporated to afford the corresponding α-diazoketone as a brown oil. The residue was chromatographed on silica using 2.5% EtOAc-toluene as eluent to give a yellowish solution in 77% yield which crystallized upon standing. Spectral data were in agreement with previously published data [17].

HBr (31 ml, 47%) was added dropwise under cooling at -10°C to a solution of the α-diazoketone (12.46 g, 34.8 mmol) in MeOH-$CHCl_3$ (1:2, 75 ml). After about 10 min under stirring the evolution of N_2 ceased and the aqueous phase was extracted with $CHCl_3$ (3 x 25 ml). The combined organic layers were washed with aq. saturated $NaHCO_3$ to pH 7, with brine, dried over Na_2SO_4 and evaporated to afford **6** as an oil which gave yellowish crystals (13.74 g, 33.4 mmol) in 96% yield. ^{1}H NMR δ 7.39 (m, 10 H, Ph), 7.19 (d, J = 2 Hz, 2 H, H-2; H-6), 6.83 (t, J = 2 Hz, 1 H, H-4), 5.06 (s, 4 H, PhCH_2O), 4.37 (s, 2 H, CH_2Br).

3-(3,5-Dibenzyloxybenzoyl)propanoic acid (7)

Compound **6** (13.73 g, 33.4 mmol) was dissolved in anhydrous THF (50 ml) and added dropwise to a solution prepared from diethyl malonate (10.2 ml, 67.1 mmol), anhydrous THF (25 ml) and NaH (1.52 g, 41.4 mmol). The mixture was refluxed for 2 hrs and then evaporated *in vacuo*.

The residual crude diester was hydrolyzed by refluxing with KOH (28.50 g) in EtOH-H_2O (2:1, 120 ml) for 1 h. The solution was concentrated, diluted with H_2O (50 ml), acidified with 2 N HCl and extracted with ether (3 x 200 ml). The ether layer was dried over Na_2SO_4, evaporated under reduced pressure and the crude malonic acid derivative was decarboxylated by heating at 150°C for 0.5 h or

until no more CO_2 was generated. The red coloured residue was recrystallized from EtOAc using charcoal giving **7** in 65% yield (8.41 g, 21.6 mmol), calculated from **6**. Mp and spectral data were in agreement with previously published data [18].

Methyl 4-(3,5-dihydroxyphenyl)butanoate (8)

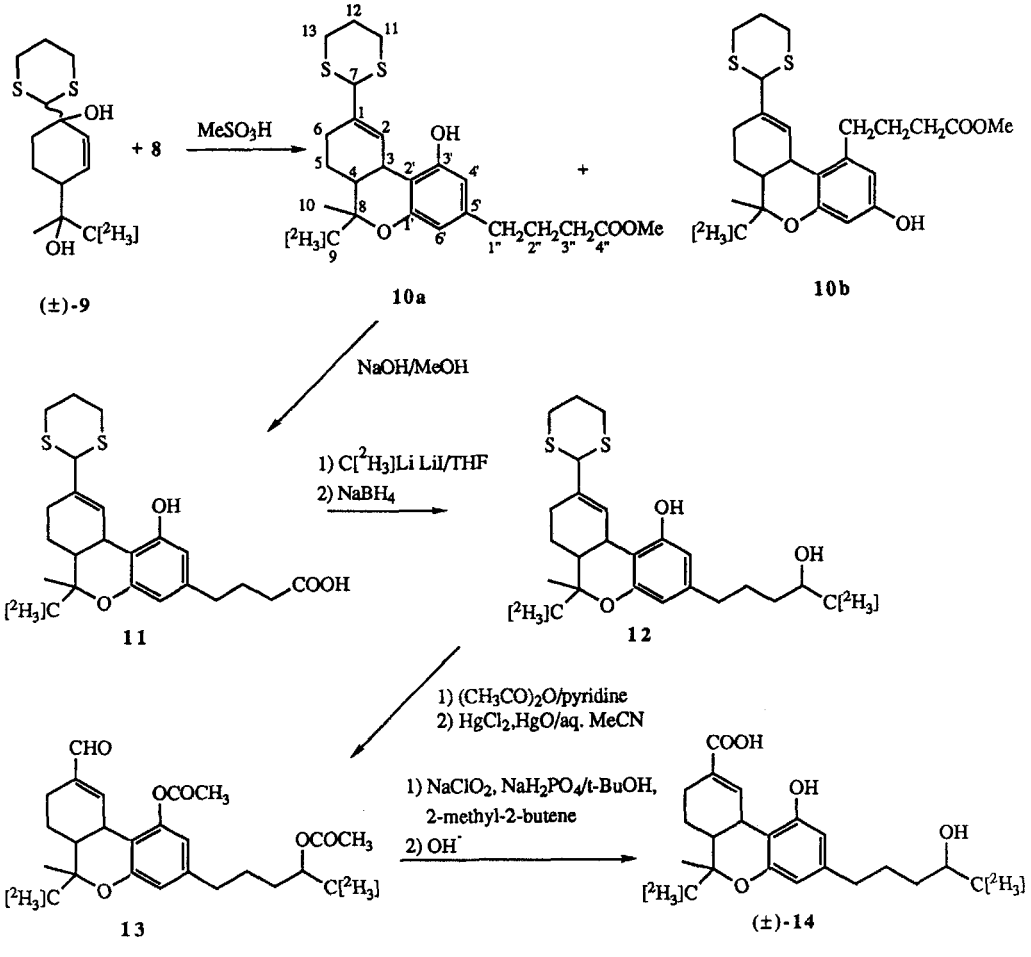
$Pd(OH)_2$ (0.24 g, 20% on charcoal) was added to a suspension of **7** (1.56 g, 4.0 mmol) in EtOAc and glacial acetic acid (1:1, 160 ml) and the mixture was hydrogenated at room temperature and 1 atm. The catalyst was filtered off after 24 hrs and the solvent evaporated.

The resulting crude mixture was dissolved in conc. H_2SO_4 and MeOH (1:5, 8 ml) and stirred for 5 hrs at room temperature. The reaction mixture was poured onto ice water (30 ml) and extracted with EtOAc (3 x 50 ml). The combined organic layers were washed with aq. saturated $NaHCO_3$ to pH 7, with brine, dried over Na_2SO_4 and evaporated to dryness. The residue was dissolved in EtOAc and evaporated on silica before being chromatographed with EtOAc-toluene (1:4) as eluent to give compound **8** in 66% yield, calculated from **7**. Spectral data were in agreement with previously published data [18].

*(±)-[$^2H_3$]-Methyl-[5",7-bisnor-1-(1,3-dithianyl)]-Δ^1-tetrahydrocannabinol-4"-oate (**10a**; see scheme 2)*

All glassware was carefully dried before use. Compound **8** (0.92 g, 4.38 mmol) was dissolved in anhydrous CH_2Cl_2 and anhydrous benzene (2:3 v/v, 165 ml) with heating to 45°C. Crystalline **9** (1.335 g, 4.82 mmol) and 0.1 eq $MeSO_3H$ were added while stirring under N_2. The reaction was quenched with H_2O (50 ml) after 6 hrs, and washed with 5% aq. $NaHCO_3$ to neutral pH. The aqueous layer was extracted with ether (3 x 75 ml). The combined organic layers were washed with brine, dried over Na_2SO_4 and evaporated *in vacuo*. The crude mixture was chromatographed on silica with ether-petroleum ether (1:2). The fractions containing mainly the isomeric **10a** and **10b** were pooled, evaporated to dryness, dissolved in 5% MeCN-CCl_4 and filtered through a Bond Elut extraction cartridge (Si, 3 ml, Analytichem International). The eluate was chromatographed on a Lobar column (size B) with 5% MeCN-CCl_4 (2 ml/min) using a low pressure pump. The yield of **10a** and **10b** by this procedure was 15% of each compound. ^1H NMR **10a** δ 6.94 (d, 1 H, H-2), 6.24 (d, 1 H, H-6'), 6.11 (d, 1 H, H-4'), 5.11 (s, OH), 4.58 (s, 1 H, H-7), 3.66 (s, 3 H, -COOC***H*****$_3$**), 3.27 (br d, J = 10 Hz, 1 H, H-3), 2.80 - 3.00 (m, 4 H, H-11 and H-13), 2.48 (t, J = 7

Hz, 2 H, H-1"), 2.31 (t, J = 7 Hz, 2 H, H-3"), 1.89 (kv, J = 7 Hz, 2 H, H-2"), 1.70 (t, J = 10 Hz, 1 H, H-4), 1.41 (s, 2 H, H-10), 1.08 (s, 1 H, H-9); ^{13}C NMR **10a** δ 174.1 (C-4"), 155.0 (C-1'), 154.5 (C-3'), 141.3 (C-5'), 135.2 (C-1), 129.5 (C-2), 110.1 (C-6'), 108.6 (C-2'), 107.6 (C-4'), 77.2 (C-8), 53.4 (C-7), 51.5 (-COO*C*H$_3$), 45.0 (C-4), 34.6 (C-3"), 33.8 (C-3), 33.4 (C-1"), 31.6 (C-11; C-13), 27.8 (C-6), 27.4 (C-10), 26.0 (C-2"), 25.5 (C-12), 24.7 (C-5), 19.2 (C-9); MS m/z 452 ([M$^+$]+H). The isotopic distribution according to mass spectrometry was, m/z (449-452): 450, 4.5%; 451, 1.9%; 452, 93.6%.



Scheme 2: Preparation of (±)-[^{2}H$_6$]-4"-hydroxy-Δ^1-THC-7-oic acid (**14**)

(±)-[$^2H_3$]-[5",7-bisnor-1-(1,3-dithianyl)]-Δ^1-tetrahydrocannabinol-4"-oic acid (11)

10a (0.195 g, 0.43 mmol) was dissolved in 2 N NaOH (2.85 ml) and MeOH-H_2O (4:1, 35 ml) and refluxed for 1 h. The solution was concentrated and extracted with ether (3 x 30 ml), the aqueous phase acidified with 2 N HCl to pH 5 and extracted with ether (3 x 50 ml). The latter organic layers were combined, dried over Na_2SO_4 and evaporated under reduced pressure to give **11** in 92% yield (0.173 g, 0.40 mmol). ^1H NMR **11** δ 6.95 (s, 1 H, H-2), 6.24 (d, J = 1.6 Hz, 1 H, H-6'), 6.13 (d, J = 1.6 Hz, 1 H, H-4'), 4.59 (s, 1 H, H-7), 3.28 (br d, J = 11 Hz, 1 H, H-3), 2.80-3.00 (m, 4 H, H-11; H-13), 2.50 (t, J = 7 Hz, 2 H, H-3"), 2.35 (t, J = 7 Hz, 2 H, H-1"), 2.05-2.15 (m, H-12), 1.90 (m, J = 7 Hz, H-2"), 1.70 (t, J = 11 Hz, 1 H, H-4), 1.40 (s, 2 H, H-10), 1.08 (s, 1 H, H-9); ^{13}C NMR **11** δ 178.7 (C-4"), 155.0 (C-1'), 154.6 (C-3'), 141.1 (C-5'), 135.1 (C-1), 129.6 (C-2), 110.0 (C-6'), 108.6 (C-2'), 107.7 (C-4'), 53.4 (C-7), 45.0 (C-4), 34.4 (C-3"), 33.8 (C-3), 33.1 (C-1"), 31.6; 31.4 (C-11; C-13), 27.8 (C-6), 27.4 (C-10), 25.7 (C-2"), 25.5 (C-12), 24.7 (C-5), 19.2 (C-9); MS m/z 438 ([M$^+$]+H). The isotopic distribution according to mass spectrometry was, m/z (435-438): 437, 0.1%; 438, 99.8%.

(±)-[$^2H_6$]-[4"-hydroxy-7-nor-1-(1,3-dithianyl)]-Δ^1-tetrahydrocannabinol (12)

Compound **11** (0.272 g, 0.62 mmol) was dissolved in anhydrous THF (10 ml) and cooled to 0°C. After 5 min C[$^2H_3$]Li·LiI (8.1 ml, 4.05 mmol, 0.5 M in ether) was added quickly and the solution was kept at 0°C for 2.5 hrs while stirring. The reaction mixture was poured onto portions of aq. saturated NH_4Cl (180 ml) under vigorous stirring, extracted with ether (3 x 200 ml), dried over Na_2SO_4 and evaporated *in vacuo*.

$NaBH_4$ (0.190 g, 3.80 mmol) was added immediately to a solution of crude [$^2H_6$]-[4"-oxo-7-nor-1-(1,3-dithianyl)]-Δ^1-THC in MeOH (10 ml) while stirring. After 15 min the reaction mixture was poured onto ice water (20 ml), neutralized by dropwise addition of 2 N HCl and extracted with ether (3 x 30 ml). The etheral solution was dried over Na_2SO_4 and evaporated *in vacuo* to give **12** in 59% yield calculated from **11** (0.145 g, 0.33 mmol) after purification on a Lobar column, as previously described, with EtOAc-toluene (1:1) as eluent. ^1H NMR **12** δ 6.96 (d, J = 1 Hz, 1 H, H-2), 6.24 (d, J = 1 Hz, 1 H, H-6'), 6.12 (d, J = 1 Hz, 1 H, H-4'), 5.55 (br s, 1 H, OH), 4.59 (s, 1 H, H-7), 3.80 (br t, 1 H, H-4"), 2.78-3.00 (m, 4 H, H-11; H-13), 2.37-2.50 (m, 4 H, H-1"; H-6), 2.02-2.14 (m, H-5), 1.70 (td, 1 H, H-4), 1.40 (s, H-10), 1.08 (s, 1 H, H-9); ^{13}C **12** NMR δ 154.9 (C-1'), 154.7 (C-3'), 142.3 (C-5'), 135.0 (C-1), 129.7 (C-2), 109.8 (C-6'), 108.4 (C-2'), 107.6 (C-4'),

77.3 (C-8), 68.1 (C-4"), 53.5 (C-7), 45.0 (C-4), 38.7 (C-3"), 35.3 (C-1"), 33.9 (C-3), 31.6 (C-11; C-13), 27.8 (C-6), 27.5 (C-10), 27.0 (C-2"), 25.5 (C-12), 24.7 (C-5), 19.2 (C-9); MS m/z 584 (M^+). The isotopic distribution according to mass spectrometry was, m/z (578-584): 579, 0.1%; 581, 1.5%; 582, 8.0%; 583, 27.6%; 584, 62.8%.

(±)-[$^2H_6$]-(3',4"-diacetoxy-1-formyl-7-nor)-Δ^1-tetrahydrocannabinol (13)

12 (0.106 g, 0.24 mmol) was treated with acetic acid anhydride (3.25 ml) in pyridine (6.50 ml) at room temperature over night. The reaction mixture was poured onto ice water (50 ml) and extracted with ether (3 x 75 ml). The combined etheral layers were washed with small portions of 2 N HCl, and then with aq. saturated $NaHCO_3$ to neutral pH, with brine, dried over Na_2SO_4 and by azeotropic distillation with benzene, giving an oil in 90% yield (0.113 g, 0.22 mmol). ^1H NMR **diacetylated 12** δ 6.60 (d, J = 1.5 Hz, 1 H, H-2), 6.54 (d, J = 1.7 Hz, 1 H, H-6'), 6.24 (d, J = 1.7 Hz, 1 H, H-4'), 4.89 (br t, 1 H, H-4"), 3.12 (br d, J = 11 Hz, 1 H, H-3), 2.78-3.00 (m, 4 H, H-11; H-13), 2.34-2.46 (m, H-6), 2.36 (s, Ar-OCOC***H*****$_3$**), 2.03 (s, R-OCOC***H*****$_3$**), 1.40 (s, H-10), 1.07 (s, H-9); ^{13}C NMR **diacetylated 12** δ 170.9 (R-O*C*OCH$_3$), 169.0 (Ar-O*C*OCH$_3$), 154.6 (C-1'), 149.4 (C-3'), 142.2 (C-5'), 136.8 (C-1), 128.6 (C-2), 115.3 (C-6'), 114.3 (C-2'*), 114.1 (C-4'*), 77.3 (C-8), 70.7 (C-4"), 52.4 (C-7), 44.7 (C-4), 35.4 (C-3"), 35.1 (C-1"), 34.2 (C-3), 31.8; 31.7 (C-11; C-13), 28.1 (C-6), 27.3 (C-10), 26.6 (C-2"), 25.6 (C-12), 24.6 (C-5), 21.4 (Ar-OCO*C*H$_3$), 21.4 (R-OCO*C*H$_3$), 19.2 (C-9) (* interchangeable shifts); MS m/z 524 (M^+). The isotopic distribution according to mass spectrometry was, m/z (518-524): 521, 0.1%; 522, 0.9%; 523, 5.9%; 524, 93.0%.

Diacetylated 12 (0.105 g, 0.20 mmol) in 80% aq. MeCN (10 ml) was added at room temperature while stirring to a solution of $HgCl_2$ (0.10 g, 0.47 mmol) in 80% aq. MeCN (3 ml) resulting in the formation of a white precipitate. Red HgO (0.05 g, 0.24 mmol) was added and the orange suspension was heated under reflux for 2 hrs under N_2. After cooling the suspension was filtered through Celite in a Bond Elut extraction cartridge (Si) and washed with CH_2Cl_2. The combined organic layers were washed sequentially with 5 M aq. ammonium acetate, H_2O, and brine, and dried over Na_2SO_4 to give **13** (0.08 g, 0.18 mmol) in 90% yield. ^1H NMR **13** δ 9.46 (s, 1 H, H-7), 7.38 (d, J = 1.7 Hz, 1 H, H-2), 6.59 (d, J = 1 Hz, 1 H, H-6'), 6.47 (d, J = 1 Hz, 1 H, H-4'), 4.90 (br t , 1 H, H-4"), 3.35 (br d, J = 11 Hz, 1 H, H-3), 2.31 (s, Ar-OCOC***H*****$_3$**), 2.03 (s, R-OCOC***H*****$_3$**), 1.75 (td, 1 H, H-4), 1.45 (s, H-10), 1.14 (s, H-9); ^{13}C NMR **13** δ 193.9 (C-7), 170.8 (R-O*C*OCH$_3$),

168.6 (Ar-O*C*OCH$_3$), 154.7 (C-1'), 151.9 (C-2), 149.2 (C-3'), 143.0 (C-5'*), 141.6 (C-1*), 115.7 (C-6'), 114.2 (C-4'), 112.6 (C-2'), 77.2 (C-8), 70.6 (C-4"), 44.8 (C-4), 35.6 (C-3), 35.4 (C-3"), 35.2 (C-1"), 27.3 (C-10), 26.6 (C-2"), 23.5 (C-5), 22.3 (C-6), 21.4 (Ar-O*C*OCH$_3$), 21.2 (R-O*C*OCH$_3$), 19.1 (C-9) (* interchangeable shifts).

(±)-[$^2H_6$]-4"-Hydroxy-Δ^1-tetrahydrocannabinol-7-oic acid (14)

80% $NaClO_2$ (0.355 g) and NaH_2PO_4 (0.338 g) were dissolved in H_2O (9.1 ml) and added in portions during 0.5 h to a solution of **13** (0.146 g, 0.34 mmol) in *t*-butanol (18.2 ml) and 2-methyl-2-butene (18.2 ml). The two phase mixture was stirred at room temperature till the reaction was complete according to TLC. The reaction mixture was concentrated and dissolved in CH_2Cl_2-H_2O (1:1, 70 ml). The layers were separated and the aqueous phase was extracted with CH_2Cl_2 (2 x 50 ml). The combined organic layers were washed with 0.1 M HCl, with H_2O, dried over Na_2SO_4, and evaporated *in vacuo* to give an oil. ^{1}H NMR **diacetylated 14** δ 7.75 (d, J = 2 Hz, 1 H, H-2), 6.57 (d, J = 1.6 Hz, 1 H, H-6'), 6.46 (d, J = 1.6 Hz, 1 H, H-4'), 4.90 (m, 1 H, H-4"), 3.26 (br d, J = 11 Hz, 1 H, H-3), 2.35-2.65 (m, H-1"; H-6), 2.32 (s, 3 H, Ar-OCOC*H*$_3$), 2.03; 1.98-2.08 (s; m, 4 H, R-OCOC*H*$_3$; H-5), 1.73 (t, 1 H, H-4), 1.44 (s, H-10), 1.12 (s, H-9); ^{13}C NMR **diacetylated 14** δ 172.2 (C-7), 170.9 (R-O*C*OCH$_3$), 168.9 (Ar-O*C*OCH$_3$), 154.6 (C-1'), 149.2 (C-3'), 143.0 (C-2), 142.7 (C-5'), 130.0 (C-1), 115.5 (C-6'), 114.3 (C-4'), 112.9 (C-2'), 70.7 (C-4"), 44.0 (C-4), 35.4 (C-3"), 35.2 (C-1"), 35.0 (C-3), 27.3 (C-10), 26.5 (C-2"), 24.8 (C-6), 24.1 (C-5), 21.4 (R-OCO*C*H$_3$), 21.1 (Ar-OCO*C*H$_3$), 19.2 (C-9); MS m/z 464 (M$^+$; **methyl ester of diacetylated 14**). The isotopic distribution according to mass spectrometry was, m/z (458-464): 459, 0.5%; 460, 6.2%; 461, 2.1%; 462, 5.4%; 463, 6.4%; 464, 79.4%.

The crude **diacetylated 14** was dissolved in a solution of KOH (0.598 g) in EtOH (10.2 ml) and H_2O (0.6 ml) and stirred for 1.5 hrs at room temperature. The reaction mixture was concentrated, diluted with H_2O (20 ml) and extracted with ether (3 x 25 ml). The aqueous phase was acidified to pH 5 with 2 N HCl and extracted with ether (3 x 25 ml). The latter ether extracts were combined, dried over Na_2SO_4 and evaporated to dryness to give **14** (0.058 g, 0.34 mmol) in 47% yield calculated from **13**. Compound **14** was filtered through a Bond Elut cartridge (C18) and purified on a Lobar column (RP-18, Size B, 2 ml/min) using MeCN-MeOH-H_2O (2:2:1) as eluent. ^{1}H NMR **14** δ 8.17 (br s, 1 H, H-2), 6.55 (br s, OH), 6.21 (s, 1 H, H-6'), 6.19 (s, 1 H, H-4'), 3.79 (t, 1 H, H-4"), 3.36 (br d, J = 10.5 Hz, 1 H, H-3), 2.30-2.60 (m, H-1"; H-6), 1.90-2.05 (m, 1 H, H-5), 1.41

(s, H-10), 1.08 (s, H-9); ^{13}C NMR **14** δ 172.4 (C-7), 155.0 (C-1'*), 154.8 (C-3'*), 145.5 (C-2), 142.7 (C-5'), 128.5 (C-1), 109.6 (C-6'), 107.8 (C-4'), 107.0 (C-2'), 77.1 (C-8), 68.3 (C-4"), 44.3 (C-4), 38.3 (C-3"), 35.2 (C-1"), 34.8 (C-3), 27.4 (C-10), 26.7 (C-2"), 25.2 (C-6), 24.2 (C-5), 19.0 (C-9) (* interchangeable shifts); MS 524 (M^+; **silylated methyl ester of 14**). The isotopic distribution according to mass spectrometry was, m/z (518-524): 521, 0.2 %; 522, 1.2 %; 523, 7.7 %; 524, 90.9 %.

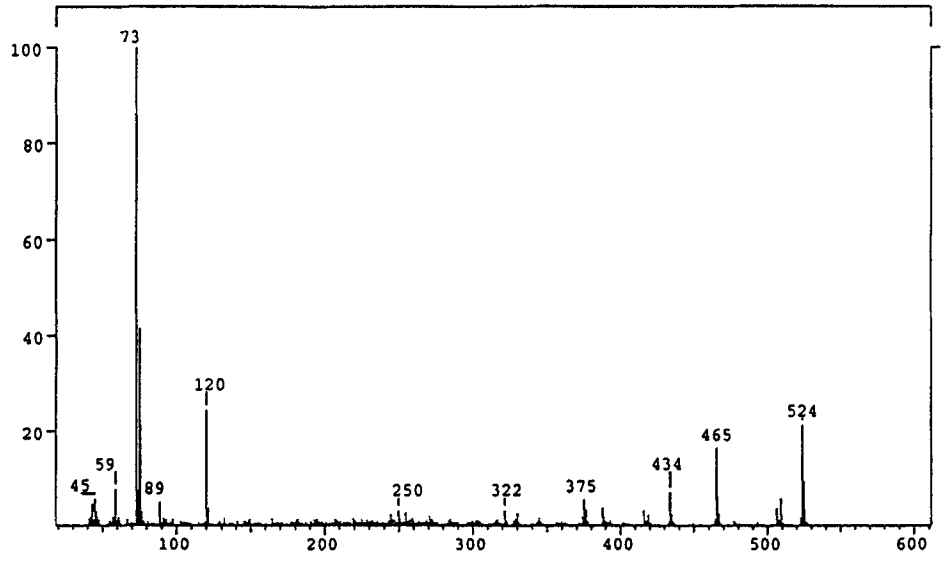


Fig 3: Mass spectrum of the silylated methyl ester of [$^2H_6$]-4"-Hydroxy-Δ^1-tetrahydrocannabinol-7-oic acid (**14**).

RESULTS AND DISCUSSION

4"-hydroxy-Δ^1-THC-7-oic acid (**4**) was first identified in rabbit as a urinary metabolite of Δ^1-THC by Nordqvist *et al.* [19]. Its formation has also been reported in guinea-pig and mouse [1]. In man, 4"-hydroxy-Δ^1-THC-7-oic acid is the third most abundant metabolite identified so far, corresponding to about 5% of the radioactivity in urine after an oral dose of [^{3}H]-Δ^1-THC [2].

Over the years synthetic methods have been developed to provide metabolites in the Δ^6-series or Δ^1-THC metabolites functionalized either in the terpene position or in the aromatic side-chain [8]. However, the only metabolites synthesized in the Δ^1-series with functional groups both in the terpene portion and the aromatic side chain are 2",7-dihydroxy-Δ^1-THC; 3",7-dihydroxy-Δ^1-THC and

recently 4",5"-bisnor-Δ^1-THC-7,3"-dioic acid [12, 20-21]. The terpene synthon has been used in the syntheses in order to achieve this. The double bond in the Δ^1-position is easily isomerized to the more thermodynamically stable Δ^6-position which causes severe limitations in the synthetic procedures available. However, the 1,3-dithiane masking group prevents this isomerization process [22].

This paper describes the first synthesis of [$^2H_6$]-labelled 4"-hydroxy-Δ^1-THC-7-oic acid (**14**). The sequence used is based on the method previously described for the synthesis of unlabelled and labelled Δ^1-THC-7-oic acid [9, 22] and 4",5"-bisnor-Δ^1-THC-7,3"-dioic acid [12], and extends the versatility of the general procedure used.

The aromatic moiety (**8**) was prepared in a somewhat modified procedure from the sequence reported by Ohlsson *et al.* [18]. The sequence described here includes protection of the phenolic groups in 3,5-dihydroxybenzoate by dibenzylation and formation of the corresponding acid chloride which is further converted to the corresponding α-diazoketone. Formation of the bromo acetophenone derivative (**6**) was accomplished by treatment with HBr, which was condensed with diethyl malonate to give the corresponding dicarboxylic ethyl ester. Saponification with KOH and decarboxylation of the corresponding dicarboxylic acid, resulting in lengthening of the alkyl chain, was followed by deprotection and reduction in a one step reaction by catalytic hydrogenation with $Pd(OH)_2$ on charcoal at atmospheric pressure. The latter reaction resulted not only in the desired **8**, after esterification but also in the formation of 4-hydroxy-(3,5-dihydroxyphenyl)butanoic acid to some extent. Other by-products that were identified during the process of optimizing the conditions were the ethyl ester of **8** and a non aromatic compound. A three step reaction sequence including Clemmensen reduction, esterification and debenzylation with palladium on charcoal was also tried but did not prove to be useful in our hands. The overall yield in the synthesis of **8** from 3,5-dihydroxybenzoic acid was 15%.

The details of the synthesis of deuterium labelled terpene synthon have previously been reported [9]. Deuterium was introduced non stereospecifically into the molecule by using [$^2H_3$]-MeMgI in the conversion of the ketone to the corresponding tertiary alcohol. The incorporation was 95.1% (m/z 295) according to mass spectrometry.

The crucial step in the synthesis, as previously discussed in the synthesis of 4",5"-bisnor-Δ^1-THC-7,3"-dioic acid [12], is the condensation between the terpene synthon (**9**) and the aromatic moiety (**8**). The yield of **10a** was 15%. **10b**, where the condensation took place *ortho* rather than *para* to the side-chain on the aromatic ring, was formed in equal amounts. The two compounds were readily

separated using open bed liquid chromatography in combination with low pressure chromatography and a Lobar column.

In our laboratory the use of an optically active terpene synthon, 4-isopropenyl-2-cyclohexen-1-one, in the condensation with **8** afforded (-)-**10a** in 15% yield. The improved results (yield 43%) previously reported from the condensation between olivetol and 4-isopropenyl-2-cyclohexen-1-one in order to obtain (-)-Δ^1-THC-7-oic acid where the reaction was performed in the presence of BF_3-Et_2O and anhydrous $MgSO_4$ and by lowering the temperature to -63°C could not be accomplished using **8** [23].

Following purification, **10a** was hydrolyzed and treated with [$^2H_3$]-MeLi·LiI in THF at 0°C, in order to introduce an additional three deuterium atoms into the molecule. In the preparation of the corresponding methyl ketone sequential treatment of **10a** with MeLi and Me_3SiCl was tried. However, quenching the reaction with excess Me_3SiCl in order to improve the yield, as described by Rubottom *et al.* only resulted in decomposition of the formed product [24]. Instead, the reaction mixture was poured onto portions of NH_4Cl under vigorous stirring. The formed compound, [$^2H_6$]-[4"-oxo-7-nor-1-(1,3-dithianyl)]-Δ^1-THC, is unstable and were therefore immediately reduced to the corresponding secondary alcohol (**12**) [17]. Some of the peaks in the ^{13}C NMR spectrum appear as doublets representing the two diastereomers of **12**. The method recently described by Ahn *et al.* showing improved yields by using Ce(III)Cl_3 in THF at -78°C and CH_3Li in the formation of the methyl ketone, was also tried but was not successful in the present case [25]. Compound **13** was prepared by the reaction of **12** with acetic acid anhydride in pyridine (2:1) and hydrolyzed using red HgO and $HgCl_2$ in aq. MeCN. The aldehyde (**11**) was oxidized using $NaClO_2$ in the presence of 2-methyl-2-butene as a hypochlorous scavenger using the method for oxidation described by Siegel *et al.* [26], followed by hydrolysis under alkaline conditions [27] resulting in the desired (±)-[$^2H_6$]-4"-hydroxy-Δ^1-THC-7-oic acid (**14**). The formation of two diastereomers due to the incorporation of deuterium in the geminal methyl groups is represented in the ^1H NMR spectrum by two singlets appearing at δ_H 1.08 and 1.41 ppm. In order to establish the correct shift values for the aromatic protons, INEPT long range technique [28] in combination with FLOCK experiments [29] were used in addition to the regularly used NMR techniques. Irradiation of the phenolic group in 3' position in the INEPT long range experiment gave a response confirming this peak coming from C-4'. The peak at 109.6 ppm therefore originates from C-6'. A comparison with previously performed NOE difference experiments [30] on Δ^1-THC-7-oic acid also confirms the above assignments. Irradiation

of the phenolic group in 3' position (8.42 ppm) then resulted in a positive NOE effect for the signal at 6.30 ppm, as well as a smaller positive NOE for the H-2 signal at 8.40 ppm, and with no effect seen for the peak at 6.18 ppm.

The mass spectral analysis of the silylated methyl ester of **14**, see figure 3, gives a molecular ion at m/z 524. Other diagnostic fragments are m/z 465 and 434 corresponding to [M^+-$COOCH_3$] and [M^+-HOTMS].

In the present study, six deuterium atoms have been introduced into the molecule. Synthesis of the unlabelled 4"-hydroxy-Δ^1-THC-oic acid metabolite may be performed using the same methods as described here. For this purpose the unlabelled terpene synthon, first reported in a short communication by Uliss *et al.* [22], could be used in the condensation with compound **8** in order to synthesize **4**. Minor modifications in the synthesis also permit the formation of other 7-substituted metabolites in labelled or unlabelled form, such as 5"-nor-Δ^1-THC-4",7-dioic acid, identified in man or 4",7-dihydroxy-Δ^1-THC.

Although the overall yield is low (3%), due to the large number of reaction steps and the involvement of a reaction which gives extremely poor yield, i.e. the condensation between the terpene synthon (**9**) and the aromatic moiety (**8**), the synthetic approach has the advantage of being versatile in combination with the ease of labelling with different isotopes (^2H, ^3H or ^{14}C). The metabolites obtained by this procedure are racemic mixtures. However, optically active metabolites should be possible to achieve by using an optically active terpene synthon in the condensation reaction. The synthesis of optically active terpene synthons which permit functional groups in 7-position have previously been described [23, 31-32].

The present synthesis is significant in that it provides an additional standard useful in the testing procedures for cannabis abuse. Other deuterated metabolites in the Δ^1-series with a functional group in 7-position which have been synthesized are [$^2H_3$]-, [$^2H_6$]- and [$^2H_{10}$]-Δ^1-THC-7-oic acid, [$^2H_5$]-4",5"-bisnor-Δ^1-THC-7,3"-dioic acid and [$^2H_9$]-7-OH-Δ^1-THC [9-12, 33]. According to a report by Joern an increased linearity was achieved in the analysis of Δ^1-THC-7-oic acid when Δ^1-THC-7-oic acid incorporated with six deuterium atoms was used as an internal standard, compared to the [$^2H_3$] homologue [34].

Evaluating the concentration of Δ^1-THC-7-oic acid together with other acidic metabolites such as 4"-hydroxy-Δ^1-THC-7-oic acid, should give an improved accuracy in the analysis of illicit use of cannabis. The urinary metabolites manifest a considerably longer time course and should therefore

provide a good indicator of previous smoking compared to analysis of THC in plasma. Δ^1-THC-7-oic acid, the major urinary Δ^1-THC metabolite and the most frequently studied metabolite, has been detected in urine using GC/MS (SIM) up to 14 days after oral administration of marijuana [35]. It remains to be determined what role acidic metabolites other than Δ^1-THC-7-oic acid may have in the analysis of biological specimens.

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