# Synthesis of the Major Mammalian Metabolites of THCV

Francesco Tinto,*[,†] Rosaria Villano,[†] Magdalena Kostrzewa,[†,‡] Alessia Ligresti,[†] Hannah Straker,[§] and Emiliano Manzo[†]

† Institute of Biomolecular Chemistry, National Research Council of Italy, Pozzuoli (NA), Italy

‡ Institute of Genetics and Biophysics, National Research Council of Italy, Naples, Italy

§ GW Pharmaceuticals, Kent Science Park, Sittingbourne, Kent, ME9 8AG, United Kingdom

**ABSTRACT:** A simple synthesis of the major oxidized metabolites in mammalian tissues of (-)-$\Delta^9$-tetrahydrocannabivarin (THCV) (**1**) has been accomplished by kinetic studies of allylic oxidation using $SeO_2$ on botanically derived THCV with the aim to yield primary and secondary allylic alcohols concurrently. This synthetic approach led to the preparation of numerous THCV derivatives, including two new compounds, 8α-hydroxy-$\Delta^9$-tetrahydrocannabivarin (**2**) and 8β-hydroxy-$\Delta^9$-tetrahydrocannabivarin (**3**), and the known compounds, 11-hydroxy-$\Delta^9$-tetrahydrocannabivarin (**4**) and $\Delta^9$-tetrahydrocannabivarin-11-oic acid (**5**), without affecting the C-10a stereogenic center in the natural precursor, and without formation of tricyclic dibenzopyrane derivatives. This simple synthetic methodology could be useful to investigate the pharmacological role of THCV metabolites at, among others, the endocannabinoid CB1 and CB2 receptors for which THCV reportedly acts as, respectively, a neutral antagonist and partial agonist.

After the discovery of the primary active constituent of marijuana, (−)-*trans*-$\Delta^9$-tetrahydrocannabinol (THC) in 1964,[1] a minor cannabinoid (−)-*trans*-$\Delta^9$-tetrahydrocannabivarin (THCV) (**1**)[2] was found in cannabis tincture in 1970.[3] The only difference between these two cannabinoids was the presence of a propyl group linked to the aromatic moiety in THCV instead of a pentyl group in THC. Five different numbering systems[4] have been used for the phytocannabinoids in general but only two are used for the benzopyran stuctures.[5] Here we employed the dibenzopyran numbering the most widespread system used for THCV (**1**) and the subsequent metabolites thereof (**2-5**) as shown in Scheme 1. Since its discovery, the pharmacology of THCV has been studied in order to consider its potential clinical use.[6] This phytocannabinoid is capable of interacting with the endocannabinoid system via binding at the CB1 and CB2 receptors where it exerts either antagonistic or agonistic effects, depending on its concentration. THCV appears to antagonise the CB1 receptor at lower doses (3mg $kg^{-1}$ or less) whereas it produces an agonist effect at higher doses (10 mg $kg^{-1}$ or more) in vivo.[7] THCV behave also in vitro as a potent competitive antagonist of cannabinoid derivatives such as CP55940 and *R*-(+)-WIN55212.[8] As regards to CB2 receptors, THCV has been defined as partial agonist in vitro[7] and currently there is limited knowledge about its behavior on CB2 receptors in vivo. The CB1 receptors are found in the central nervous system (CNS) as well as many peripheral tissues[9] while CB2 receptors are found primarily outside the CNS in human spleen,[10] the tonsils,[11] leukocytes,[12] and tissues associated with the immune system.[13] CB2 receptors are not totally absent from the brain since they are expressed in microglia,[14] but their presence and role in the CNS have yet to be fully elucidated.[15] Unlike the other phytocannabinoids little is known about the pharmacokinetics of THCV in humans, although a phase II trial of THCV in patients with type II diabetes was recently completed (NCT02053272)

by GW Research Ltd. However, even less is known about the pharmacokinetics of its oxidized metabolites. Additional experiments are now required to establish whether THCV metabolites can also interact with CB1 and CB2 receptors in vitro, in various tests of functional activity, and in vivo. The availability of synthesized THCV metabolites is therefore critical for the analysis of their biological actions and, although some approaches have been reported[16,17,18,19] for their de novo synthesis, none of these have started from botanically-derived, stereochemically pure THCV. Cannabinoids are sensitive to oxidative environments and for this reason, the oxidized metabolites are often prepared by complex de novo syntheses that require expensive stereoselective reagents. With the optimization of methods of isolation and purification of products from botanical sources, and the growing expansion of *C. sativa* for medical purposes, it would be useful to design a semisynthetic method starting from natural precursors that is quicker, more efficient, and "green". We report here a simple methodology to synthesize different oxidized metabolites of THCV, including 8α-hydroxy-$\Delta^9$-tetrahydrocannabivarin (**2**), 8β-hydroxy-$\Delta^9$-tetrahydrocannabivarin (**3**) , 11-hydroxy-$\Delta^9$-tetrahydrocannabivarin (**4**), and $\Delta^9$-tetrahydrocannabivarin-11-oic acid (**5**), in a limited number of steps starting from purified THCV extracted from a THCV-rich strain of *C. sativa*.

## RESULTS AND DISCUSSION

For the preparation of the oxidized THCV metabolites (**2**-**5**), botanical purified THCV (**1**) supplied by GW Research Ltd., was converted into its 1-*O*-acetyl derivative (**6**) using $Ac_2O$ in pyridine followed by subsequent oxidation (Scheme 2). The first attempt of this oxidation was carried out on a mixture of $\Delta^8$- and $\Delta^9$-THC using an excess of $SeO_2$ at 78°C as described by Ben-Zvi *et al.*[20] When these experimental conditions were evaluated using the major cannabinoid in *C. sativa,* a complex mixture of hyperoxidized $\Delta^8$- and $\Delta^9$-THC

metabolites was obtained without achieving significant yields of the targeted THC oxidized metabolites. In order to exploit the oxidizing potential of $SeO_2$ while avoiding the formation of unwanted byproducts and increasing the overall yields, several experimental conditions were attempted to selectively oxidise THCV. Thus, oxidation of THCV with *tert*-butyl hydroperoxide (1.34 eq) and $SeO_2$ (0.55 eq) in $CH_2Cl_2$ at room temperature was evaluated, but without success. This method failed to oxidize the allylic C-11 and led to a complex mixture which confirmed the sensitivity of these molecules to the common allylic oxidation conditions. Subsequently, oxidation using $SeO_2$ in EtOH at reflux (in the absence of *tert*-butyl hydroperoxide) was attempted. This experiment was designed to avoid oxidation at the C-10a stereogenic center of (-)-$\Delta^9$-THCV at elevated temperatures and high concentrations of oxidants. Oxidation with only $SeO_2$ (10 eq) left the starting material unchanged, indicating *tert*-butyl hydroperoxide as alternative hyperoxidant for these cannabinoids. Subsequent attempts to increase the equivalents of $SeO_2$ (30 eq) proved to be too harsh. The THCV was completely transformed into unwanted double oxidized derivatives and tricyclic dibenzopyrane byproducts. (Figure 1).

Owing to the high sensitivity of the THCV structure to the oxidizing conditions observed in these attempts, the allylic oxidation reaction was modified by changing the $SeO_2$ concentration and reaction time while studying the reaction kinetics using mass spectrometry. These experiments permitted the design of an optimised methodology to produce THCV metabolites with retention of the C-10a stereocenter (**2-4,** Scheme 1). The mass spectrometry and kinetic studies were carried out on two different allylic oxidations using 15 and 30 eq of $SeO_2$ in EtOH at reflux. These reactions were monitored by taking aliquots every 30 minutes over 4 hours, followed by HRESIMS analysis. Initially, 15 eq

of $SeO_2$ was used and after 60 minutes THCV was observed unaltered. However, at 90 minutes, it was completely consumed and transformed into multiple oxidation products as assessed by TLC and HRESIMS data. The mass spectrum of this sample was consistent with the three oxidized compounds **2-4** and some byproducts with higher masses. In the second experiment using 30 equivalents of $SeO_2$, THCV was consumed after 30 minutes based on TLC and HRESIMS data, and after 60 minutes many by-products were observed. The mass spectrum did not show any notable peaks corresponding to the targeted products. The targeted products were observed with 15 equivalents of $SeO_2$ in EtOH at reflux over 75 minutes. Compound **6** was oxidised according to this optimized strategy. The $SeO_2$ was filtered and the EtOH was evaporated and the mixture extracted with $Et_2O$. Silica gel and UHPLC purifications afforded the acetylated metabolites, 11-hydroxy-$\Delta^9$-1-*O*-acetyltetrahydrocannabivarin (**7**, 10%), 8$\alpha$-hydroxy-$\Delta^9$-1-*O*-acetyltetrahydrocannabivarin (**8**, 12%), and 8$\beta$-hydroxy-$\Delta^9$-1-*O*-acetyltetrahydrocannabivarin (**9**, 20%) (Scheme 2). Subsequent saponification of the acetate groups in alkaline conditions led to metabolite 11-hydroxy-$\Delta^9$-tetrahydrocannabivarin (**4**), and the new compounds 8$\alpha$-hydroxy-$\Delta^9$-tetrahydrocannabivarin (**2**), and 8$\beta$-hydroxy-$\Delta^9$-tetrahydrocannabivarin (**3**). All compounds were characterized by NMR ($^1H/^{13}C$, COSY, and HSQC) and HRESIMS data. The THCV derivatives were confirmed to be $\Delta^9$- instead of $\Delta^8$- isomers matching the same peculiar higher chemical shifts for C-10, C-8, and H-10, and H-8 as previously described in the characterization of $\Delta^9$-THC and $\Delta^8$-THC derivatives.[21,22] The structure of compound **4** was elucidated through the HSQC spectrum showing the hydroxymethyl group at C-11 whereas the hydroxymethine group was at C-8 in compounds **2** and **3** with the hydroxy groups in $\alpha$- and $\beta$-orientations, respectively, by

close analogy with spectroscopic data of the botanical pentyl derivatives 8*α*-hydroxy-$\Delta^9$-tetrahydrocannabinol and 8*β*-hydroxy-$\Delta^9$-tetrahydrocannabinol described by Radwan *et al.*[21] In fact, the presence of a C-8 oxymethine carbon at $\delta_C$ 68.0 corresponding to a proton resonance H-8 at $\delta_H$ 4.10 in the HSQC spectrum, showed that 8*α*-hydroxy-$\Delta^9$-tetrahydrocannabivarin (**2**) is a hydroxylated $\Delta^9$-THCV derivative. The NMR data of 8*β*-hydroxy-$\Delta^9$-tetrahydrocannabivarin (**3**) were similar to those of compound **2** except for the chemical shifts of H-8 at $\delta_H$ 4.30 and C-8 at $\delta_C$ 71.5. The higher frequency shifts indicated the *β*-orientation as reported for the 8*α*- and 8*β*-hydroxy-$\Delta^9$-tetrahydrocannabinols.[21]

With the aim of obtaining $\Delta^9$-tetrahydrocannabivarin-11-oic acid (**5**)[23], compound **7** was oxidized to the corresponding aldehyde (**10**) using $MnO_2$ (Scheme 3). This mild oxidant was chosen to obtain the aldehyde (**10**) instead of directly the carboxylic acid to avoid possible by-products formation using stronger oxidants. The method led to the isolation and characterization of this important compound that can be useful for future derivatizations at C-11. The subsequent Pinnick oxidation[24] oxidized aldehyde **10** to the corresponding acetylated carboxylic acid (**11**) which gave $\Delta^9$-tetrahydrocannabivarin-11-oic acid (**5**) in high yield (98%) after hydrolysis under alkaline conditions.

In conclusion, we developed a simple methodology for the synthesis of THCV oxidized metabolites starting from botanical sources and with retention of absolute configuration. The controlled approach uses an optimized allylic oxidation methodology as key step and permitted synthesis of four metabolites **2**-**5**, two of which 8*α*-hydroxy-$\Delta^9$-tetrahydrocannabivarin (**2**) and 8*β*-hydroxy-$\Delta^9$-tetrahydrocannabivarin (**3**) are new. This method requires only three steps to access the hydroxylated derivatives and five steps for

the corresponding carboxylic acid, starting from the pure $\Delta^9$-THCV extracted from *Cannabis sativa*. This chemical approach may be used for semi-synthesis of cannabinoid derivatives to form a range of oxidation products. Moreover, it was investigated whether these compounds are able to bind CB1 and CB2 receptors. In particular, the new molecules were evaluated in parallel with THCV as reference compound, in a competition-binding assay. The binding affinities for human recombinant CB1 and CB2 receptors are collated in Table 1. Nearly all compounds displayed binding affinity for CBRs, with Ki values for CB1 spanning two orders of magnitude (1.45 to 186.8 nM), and with Ki values for CB2 spanning three orders of magnitude (17.6 to 3715.0 nM). Only 11-hydroxy-$\Delta^9$-tetrahydrocannabivarin (**4**) showed a pattern comparable to THCV with similar affinity to both subtypes, while 8$\alpha$-hydroxy-$\Delta^9$-tetrahydrocannabivarin (**2**) and $\Delta^9$-tetrahydrocannabivarin-11-oic acid (**5**) showed higher affinity for CB1R compared to CB2R (Ki values 25.6 versus 128.2, and 186.8 versus 589.7 respectively). Moreover, the chiral metabolite 8$\beta$-hydroxy-$\Delta^9$-tetrahydrocannabivarin (**3**) was devoid of receptor affinity (Ki > 10,000 nM) suggesting distinct interactions of the two stereoisomers with the binding pocket. Further studies will elucidate how they activate cannabinoids receptors.

## EXPERIMENTAL SECTION

**General Experimental Procedures.** The structures of products and synthetic intermediates were assigned using $^1$H, $^{13}$C, COSY, HSQC, HMBC NMR spectra. $^1$H, $^{13}$C, and 2D NMR spectra were acquired by the NMR Service of the Institute of Biomolecular Chemistry of the National Council of Research (ICB-CNR) and recorded on a Bruker

DRX-600 spectrometer, equipped with a TCI CryoProbe™, fitted with a gradient along the Z-axis and on Bruker instrument ($^{1}$H 400; $^{13}$C 101 MHz). Samples for NMR spectroscopic analysis were dissolved in the appropriate solvent; spectra in $CDCl_3$: $\delta_H$ 7.26, $\delta_C$ 77.0; methanol-$d_4$: $\delta_H$ 3.34, $\delta_C$ 49.0. HRESIMS data were acquired on a Micromass Q-TOF MicroTM coupled with an HPLC Waters Alliance 2695. TLC plates (Kieselgel 60 F254) and silica gel powder (Kieselgel 60, 0.063-0.200 mm) were from Merck (Darmstadt, Germany). All the reagents and solvents were purchased from Sigma-Aldrich (Steinheim, Germany), and used without further purification.

*1-O-Acetyl-$\Delta^9$-tetrahydrocannabivarin* ***(6).*** (-)-$\Delta^9$-THCV (**1**), obtained from GW Pharmaceutical company (London, United Kingdom) (100 mg, 0.35 mmol), was dissolved in pyridine (2 mL) and $Ac_2O$ (2 mL), and the mixture was stirred for 18 hours at room temperature. The solution was poured onto iced water (20 mL) and extracted with $Et_2O$ (3x15 mL). The combined organic extracts were washed with 1N HCl, aqueous $NaHCO_3$ and brine, dried on $MgSO_4$, and filtered. Removal of the solvents under reduced pressure afforded an oily residue that by $^{1}$H NMR data were proven to be (**6**) (111 mg, 0.34 mmol, ~99%); $^{1}$H NMR ($CDCl_3$, 400 MHz) $\delta_H$ 6.55 (1H, d, J=1.6 Hz, H-2), 6.40 (1H, d, J=1.6 Hz, H-4), 5.98 (1H, q, J=1.6 Hz, H-10), 3.10-3.04 (1H, dm, J=10.9 Hz, H-10a), 2.48 (2H, t, J=7.8 Hz, H-1'), 2.28 (3H, s, H-2''), 2.14 (2H, m, H-8), 1.90 (1H, m, H-7), 1.70 (1H, m, H-6a), 1.67 (3H, s, H-11), 1.62 (2H, m, H-2'), 1.40 (3H, s, H-13), 1.39 (1H, m, H-7), 1.09 (3H, s, H-12), 0.91 (3H, t, J=7.2 Hz, H-3'); $^{13}$C NMR ($CDCl_3$, 101 MHz) $\delta_C$ 122.4 (CH, C-10), 114.6 (CH, C-4), 113.2 (CH, C-2), 44.8 (CH, C-6a), 37.2 ($CH_2$, C-1'), 33.6 (CH, C-10a), 30.5 ($CH_2$, C-8), 27.0 ($CH_3$, C-13), 24.3 ($CH_2$, C-7), 23.8

($CH_3$, C-11), 23.2 ($CH_2$, C-2'), 20.7 ($CH_3$, C-2''), 18.8 ($CH_3$, C-12), 13.3 ($CH_3$, C-3'); HRMS (ESI) *m/z* 351.2035 $[M+Na]^+$ (calcd 351.1931 for $C_{21}H_{28}O_3$).

*11-Hydroxy-$\Delta^9$-1-O-acetyltetrahydrocannabivarin* ***(7).*** Compound **6** (111 mg, 0.34 mmol) was dissolved in EtOH (5 mL), $SeO_2$ (566 mg, 5.1 mmol) was added, and the reaction was stirred at reflux for 75 minutes. The mixture was filtered and the EtOH removed under reduced pressure, the residue was diluted with $H_2O$ (20 mL) and extracted with $Et_2O$ (3x15 mL). The combined organic extracts were dried on $Na_2SO_4$, and filtered. Removal of the solvent under reduced pressure afforded a residue that was chromatographed on silica gel (30% ether/light petroleum ether) to give 61 mg of the mixture (**7**, **8**, **9**). Compound **7** was isolated with UHPLC isocratic chromatography (silica column, normal phase, eluent isopropanol: *n*-hexane 5:95 v/v; flow=3 mL/min; λ=220 nm), at retention time 16.5 min (11 mg, 0.034 mmol, 10%). TLC (50% $Et_2O$/light petroleum ether, $R_f$=0.18); $^1H$ NMR ($CDCl_3$, 400 MHz) $\delta_H$ 6.55 (1H, d, J=1.4 Hz, H-2), 6.41 (1H, d, J=1.4 Hz, H-4), 6.31 (1H, H-10), 4.02 (2H, s, H-11), 3.16-3.10 (1H, dm, J=11.4 Hz, H-10a), 2.48 (2H, t, J=7.4 Hz, H-1'), 2.28 (3H, s, H-2''), 2.26 (2H, m, H-8), 2.01-1.94 (1H, m, H-7), 1.70 (1H, m, H-6a), 1.60 (2H, m, H-2'), 1.42 (3H, s, H-13), 1.40 (1H, m, H-7), 1.09 (3H, s, H-12), 0.91 (3H, t, J=7.2 Hz, H-3'); $^{13}C$ NMR ($CDCl_3$, 101 MHz) $\delta_C$ 123.8 (CH, C-10), 114.7 (CH, C-4), 113.1 (CH, C-2), 66.4 ($CH_2$, C-11), 44.9 (CH, C-6a), 36.8 ($CH_2$, C-1'), 33.5 (CH, C-10a), 26.5 ($CH_3$, C-13), 26.1 ($CH_2$, C-8), 24.3 ($CH_2$, C-7), 23.2 ($CH_2$, C-2'), 20.7 ($CH_3$, C-2''), 18.8 ($CH_3$, C-12), 13.3 ($CH_3$, C-3'); HRMS (ESI) *m/z* 367.0705 $[M+Na]^+$ (calcd 367.1880 for $C_{21}H_{28}O_4$).

*8α-Hydroxy-$\Delta^9$-1-O-acetyltetrahydrocannabivarin* ***(8).*** Compound **8** was isolated with UHPLC isocratic chromatography (silica column, normal phase, eluent isopropanol: *n*-hexane 5:95 v/v; flow=3 mL/min; λ=220 nm), at retention time 14.5 min (13 mg, 0.042 mmol, 12%).

TLC (50% $Et_2O$/light petroleum ether, $R_f$=0.20); $^1H$ NMR ($CDCl_3$, 400 MHz) $\delta_H$ 6.55 (1H, d, J=1.4 Hz, H-2), 6.41 (1H, d, J=1.4 Hz, H-4), 6.24 (1H, H-10), 4.07 (1H, s, H-8), 2.98 (1H, dm, J=11.1 Hz, H-10a), 2.48 (2H, t, J=7.4 Hz, H-1'), 2.28 (3H, s, H-2''), 2.01 (1H, dm, J=13.6 Hz, H-7), 1.83 (1H, brs, H-6a), 1.60 (2H, m, H-2'), 1.55 (3H, m, H-11), 1.42 (3H, s, H-13), 1.38-1.33 (1H, m, H-7), 1.10 (3H, s, H-12), 0.91 (3H, t, J=7.2 Hz, H-3'); $^{13}C$ NMR ($CDCl_3$, 101 MHz) $\delta_C$ 126.4 (CH, C-10), 114.7 (CH, C-4), 113.1 (CH, C-2), 67.7 (CHOH, C-8), 40.0 (CH, C-6a), 37.1 ($CH_2$, C-1'), 34.4 (CH, C-10a), 34.3 ($CH_2$, C-7), 26.7 ($CH_3$, C-13), 23.6 ($CH_2$, C-2'), 20.4 ($CH_3$, C-2'') 20.2 ($CH_3$, C-11), 19.1 ($CH_3$, C-12), 13.3 ($CH_3$, C-3'); HRMS (ESI) *m/z* 367.0705 $[M+Na]^+$ (calcd 367.1880 for $C_{21}H_{28}O_4$).

*8β-Hydroxy-$\Delta^9$-1-O-acetyltetrahydrocannabivarin* ***(9).*** Compound **9** was isolated with UHPLC isocratic chromatography (silica column, normal phase, eluent isopropanol: *n*-hexane 5:95 v/v; flow=3 mL/min; λ=220 nm) at retention time 12.0 min (22 mg, 0.070 mmol, 20%). TLC (50% $Et_2O$/light petroleum ether, $R_f$=0.20); $^1H$ NMR ($CDCl_3$, 400 MHz) $\delta_H$ 6.55 (1H, d, J=1.4 Hz, H-2), 6.41 (1H, d, J=1.4 Hz, H-4), 6.15 (1H, brs, H-10), 4.29 (1H, s, H-8), 3.18 (1H, dm, J=11.1 HZ, 1 Hz, H-10a), 2.48 (2H, t, J=7.4 Hz, H-1'), 2.32 (1H, m, H-7), 2.28 (3H, s, H-2''), 1.81 (1H, m, H-6a), 1.77 (3H, s, H-11), 1.60 (2H, m, H-2'), 1.40 (3H, s, H-13), 1.36 (1H, m, H-7), 1.11 (3H, s, H-12), 0.91 (3H, t, J=7.2 Hz, H-3'); $^{13}C$ NMR ($CDCl_3$, 101 MHz) $\delta_C$ 125.7 (CH, C-10), 114.4 (CH, C-4), 113.0 (CH, C-2), 71.1 (CHOH, C-8), 45.2 (CH, C-6a), 36.7 ($CH_2$, C-1'), 34.8 ($CH_2$, C-7), 33.7 (CH, C-10a), 26.1 ($CH_3$, C-13), 23.0 ($CH_2$, C-2'), 20.5 ($CH_3$, C-2''), 18.5 ($CH_3$, C-12), 18.4 ($CH_3$, C-11), 13.0 ($CH_3$, C-3'); HRMS (ESI) *m/z* 367.0705 $[M+Na]^+$ (calcd 367.1880 for $C_{21}H_{28}O_4$).

*11-Hydroxy-$\Delta^9$-tetrahydrocannabivarin* ***(4).*** Compound **7** (11 mg, 0.034 mmol) was dissolved in MeOH (2 mL), 1 M NaOH (1 mL) was added and the reaction mixture was stirred

for 1 hour at r.t. The solution was acidified with 1M HCl (pH~2) and extracted with $Et_2O$ (3x5 mL). The combined organic extracts were washed with brine, dried on $Na_2SO_4$, and filtered. Removal of the solvents under reduced pressure afforded the pure compound **4** (10 mg, 0.033 mmol, 97%). TLC (50% $Et_2O$/light petroleum ether, $R_f$=0.13); $^1$H NMR ($CDCl_3$, 400 MHz) $\delta_H$ 6.67 (1H, brd, J=1.1 Hz, H-10), 6.27 (1H, d, J=1.1 Hz, H-2), 6.12 (1H, d, J=1.2 Hz, H-4), 4.03 (2H, s, H-11), 3.26 (1H, dm, J=11.4 Hz, H-10a), 2.42 (2H, m, H-1'), 2.29 (2H, m, H-8), 1.99 (1H, dm, H-7), 1.71 (1H, m, H-6a), 1.58 (2H, m, H-2'), 1.43 (1H, m, H-7), 1.42 (3H, s, H-13), 1.11 (3H, s, H-12), 0.91 (3H, t, J=7.2 Hz, H-3'); $^{13}$C NMR ($CDCl_3$, 101 MHz) $\delta_C$ 125.5 (CH, C-10), 109.5 (CH, C-4), 106.7 (CH, C-2), 66.7 ($CH_2OH$, C-11), 45.0 (CH, C-6a), 36.9 ($CH_2$, C-1'), 32.8 (CH, C-10a), 26.7 ($CH_3$, C-13), 26.0 ($CH_2$, C-8), 24.0 ($CH_2$, C-7), 23.1 ($CH_2$, C-2'), 18.5 ($CH_3$, C-12), 13.1 ($CH_3$, C-3'); HRMS (ESI) *m/z* 325.2298 $[M+Na]^+$ (calcd 325.1774 for $C_{19}H_{26}O_3$).

*8α-Hydroxy-$\Delta^9$-tetrahydrocannabivarin* ***(2).*** Compound **2** (12 mg, 0.040 mmol, 95%) was synthetized starting from compound **8** (13 mg, 0.042 mmol) with the same procedure reported for compound **4**; $^1$H NMR ($CDCl_3$, 400 MHz) $\delta_H$ 6.66 (1H, brs, H-10), 6.28 (1H, s, H-2), 6.11 (1H,d, J=1.1, H-4), 4.10 (1H, d, H-8), 3.14 (1H, dm, H-10a), 2.42 (2H, dd, J=6.6, 8.4 Hz, H-1'), 2.04 (1H, dm, H-7), 1.84 (3H, s, overlapped, H-11), 1.83 (1H, m, H-6a), 1.64 (2H, m, H-2'), 1.58 (1H, m, H-7), 1.43 (3H, s, H-13), 1.10 (3H, s, H-12), 0.92 (3H, t, J=7.2 Hz, H-3'); $^{13}$C NMR ($CDCl_3$, 101 MHz) $\delta_C$ 127.6 (CH, C-10), 109.9 (CH, C-4), 107.1 (CH, C-2), 68.0 (CHOH ***α*** form, C-8), 40.1 (CH, C-6a), 37.2 ($CH_2$, C-1'), 34.4 ($CH_2$, C-7), 34.3 (CH, C-10a), 27.1 ($CH_3$, C-13), 24.4 ($CH_2$, C-2'), 20.2 ($CH_3$, C-11), 19.3 ($CH_3$, C-12), 13.5 ($CH_3$, C-3'); HRMS (ESI) *m/z* 325.2298 $[M+Na]^+$ (calcd 325.1774 for $C_{19}H_{26}O_3$).

*8β-Hydroxy-$\Delta^9$-tetrahydrocannabivarin* ***(3).*** Compound **3** (20 mg, 0.066 mmol, 94%) was synthetized starting from compound **9** (22 mg, 0.070 mmol) with the same procedure reported for compound **4**; $^1$H NMR ($CDCl_3$, 400 MHz) $\delta_H$ 6.52 (1H, brq, J=1.5 Hz, H-10), 6.26 (1H,d, J=1.4 Hz, H-2), 6.12 (1H,d, J=1.4, H-4), 4.30 (1H, brt, J=7.0 Hz, H-8), 3.31 (1H, dm, J=10.6 Hz, H-10a), 2.42 (2H, dd, J=6.6, 8.4 Hz, H-1'), 2.35 (1H, m, H-7), 1.79 (1H, m, overlapped, H-6a), 1.78 (3H, s, H-11), 1.60 (2H, m, H-2'), 1.41 (3H, s, H-13), 1.39 (1H, m, H-7), 1.12 (3H, s, H-12), 0.91 (3H, t, J=7.2 Hz, H-3'); $^{13}$C NMR ($CDCl_3$, 101 MHz) $\delta_C$ 127.8 (CH, C-10), 109.8 (CH, C-4), 107.1 (CH, C-2), 71.5 (CHOH *β* form, C-8), 45.5 (CH, C-6a), 37.1 ($CH_2$, C-1'), 35.3 ($CH_2$, C-7), 33.6 (CH, C-10a), 26.7 ($CH_3$, C-13), 23.4 ($CH_2$, C-2'), 18.9 ($CH_3$, C-11), 18.8 ($CH_3$, C-12), 13.3 ($CH_3$, C-3'); HRMS (ESI) *m/z* 325.2298 $[M+Na]^+$ (calcd 325.1774 for $C_{19}H_{26}O_3$).

*11-Oxo-$\Delta^9$-1-O-acetyltetrahydrocannabivarin* ***(10).*** A solution of compound **7** (11 mg, 0.034 mmol) was added to anhydrous $Et_2O$ (1 mL) at 0°C and treated with activated $MnO_2$ (96%, 89mg, 1.02 mmol). The reaction mixture was stirred for 1h at 0°C and overnight at rt. The mixture was filtered through celite and the filter pad was washed with $Et_2O$. The combined filtrates were dried with $Na_2SO_4$, and removal of the solvents under reduced pressure afforded pure compound **10** (11 mg, 0.033 mmol, 94%). TLC (50% $Et_2O$/light petroleum ether, $R_f$=0.60); $^1$H NMR ($CDCl_3$, 400 MHz) $\delta_H$ 9.46 (1H, s, H-11), 6.97 (1H, brd, J=1.1 Hz, H-10), 6.59 (1H, d, J=1.1 Hz, H-2), 6.47 (1H, d, J=1.2 Hz, H-4), 5.02 (2H, m, H-8), 3.35 (1H, dm, J=11.4 Hz, H-10a), 2.51 (2H, m, H-1'), 2.30 (1H, m, H-7), 2.29 (3H, s, H-2''), 2.05 (1H, m, H-6a) 1.74 (1H, m, ), 1.61 (2H, m, H-2'), 1.43 (3H, s, H-13), 1.42 (1H, m, H-7), 1.14 (3H, s, H-12), 0.92 (3H, t, J=7.2 Hz, H-3') ; $^{13}$C NMR ($CDCl_3$, 101 MHz) $\delta_C$ 192.8 (COH, C-11), 124.7 (CH, C-10), 115.0 (CH, C-4), 113.4 (CH, C-2), 44.0 (CH, C-6a), 36.8 ($CH_2$, C-1'), 34.9 (CH, C-10a), 33.3 ($CH_2$, C-

8), 29.6 ($CH_3$, C-13), 23.5 ($CH_2$, C-2'), 22.5 ($CH_2$, C-7), 18.5 ($CH_3$, C-12), 13.0 ($CH_3$, C-3'); HRMS (ESI) *m/z* 365.1926 $[M+Na]^+$ (calcd 365.1723 for $C_{21}H_{26}O_4$).

*1-O-Acetyl-$\Delta^9$-tetrahydrocannabivarin-11-oic acid* ***(11).*** $NaClO_2$ (80% pure 5.5 mg, 0.05 mmol) was added to a stirred mixture of **10** (4 mg, 0.012 mmol), 2-methyl-2-butene (0.03 ml, 0.28 mmol) and a saturated aqueous solution of $KH_2PO_4$ (14 µL) in *t*-BuOH (500 µL). The mixture was stirred at room temperature overnight. Water was added (2 mL) and the mixture was extracted with EtOAc (3x5 mL). The organic phase was washed with brine, dried over $Na_2SO_4$, and filtered. Removal of the solvent under reduced pressure gave compound **11** (3.9 mg, 0.011 mmol, 92%); $^1H$ NMR ($CDCl_3$, 400 MHz) $\delta_H$ 7.73 (1H, brd, J=2.0 Hz, H-10), 6.57 (1H, d, J=1.1 Hz, H-2), 6.46 (1H, d, J=1.2 Hz, H-4), 3.26 (1H, dm, J=11.4 Hz, H-10a), 2.60 – 2.40 (3H, m, overlapped, H-1', H-7), 2.30 (3H, s, H-2''), 2.05 (1H, m, H-6a), 1.61 (2H, m, H-2'), 1.43 (3H, m, H-13), 1.42 (1H, m overlapped, H-7), 1.12 (3H, s, H-12), 0.92 (3H, t, J=7.2 Hz, H-3'); HRMS (ESI) *m/z* 381.1871 $[M+Na]^+$ (calcd 381.1673 for $C_{21}H_{26}O_5$).

*$\Delta^9$-Tetrahydrocannabivarin-11-oic acid* ***(5).*** Prepared from **11** (3.9 mg, 0.011 mmol) using the same procedure reported for compound **4.** The pure compound **5** was isolated with UHPLC isocratic chromatography (RP-18 analytical column, eluent MeOH:$H_2O$:TFA, 80:20:0.1 v/v; flow=1 mL/min; λ=220 nm) at retention time 9.5 min (3.47 mg, 0.011 mmol, 98%). TLC (70% $Et_2O$/light petroleum ether, $R_f$=0.28); $^1H$ NMR ($CDCl_3$, 400 MHz) $\delta_H$ 8.08 (1H, brd, J=1.6 Hz, H-10), 6.28 (1H, d, J=1.4 Hz, H-2), 6.13 (1H, d, J=1.4 Hz, H-4), 3.38 (1H, dm, J=11.1 Hz, H-10a), 2.55 (1H, m, H-7), 2.43 (2H, t, J=7.4 Hz, H-1'), 2.02 (1H, m, H-6a), 1.59 (2H, m, H-2'), 1.44 (3H, s, H-13), 1.41 (1H, m, overlapped, H-7), 1.11 (3H, s, H-12), 0.91 (3H, t, J=7.2 Hz, H-3'); $^{13}C$ NMR ($CDCl_3$, 101 MHz) $\delta_C$ 144.1 (CH, C-10), 109.4 (CH, C-4), 106.8 (CH, C-2), 43.5 (CH, C-6a), 36.8 ($CH_2$, C-1'), 33.9 (CH, C-10a), 26.1 ($CH_3$, C-13), 24.4 ($CH_2$, C-8), 23.4 ($CH_2$,

C-7), 23.3 ($CH_2$, C-2'), 18.3 ($CH_3$, C-12), 13.0 ($CH_3$, C-3'); HRMS (ESI) *m/z* 339.2953 $[M+Na]^+$ (calcd 339.1567 for $C_{19}H_{24}O_4$).

**In Vitro Pharmacological Evaluation.** *Competition Binding Assay.* Membranes from HEK-293 cells over-expressing the respective human recombinant CB1R ($B_{max}$ = 2.5 pmol/mg protein) and human recombinant CB2R ($B_{max}$ = 4.7 pmol/mg protein) were incubated with [$^3$H]-CP-55,940 (0.14 nM/Kd = 0.18 nM and 0.084 nM/Kd = 0.31nM, respectively, for CB1R and CB2R) as the high affinity ligand. Competition curves were generated by displacing [$^3$H]-CP-55,940 with increasing concentration of compounds (0.1 nM – 10 μM). Nonspecific binding was defined by 10 μM of WIN55,212-2 as the heterologous competitor ($K_i$ values 9.2 nM and 2.1 nM, respectively, for CB1R and CB2R). $IC_{50}$ values were determined for compounds showing >50% displacement at 10 μM. All compounds were tested following the procedure described by the manufacturer (Perkin Elmer, Milan, Italy). Displacement curves were generated by incubating drugs with [$^3$H]-CP-55,940 for 90 minutes at 30 ºC. $K_i$ values were calculated by applying the Cheng-Prusoff equation to the $IC_{50}$ values (obtained by GraphPad) for the displacement of the bound radioligand by increasing concentrations of the test compound. Data represent mean values of three independent experiments performed in duplicate and are expressed as the average of $IC_{50}$ and Ki (nM) ± standard deviation.

**Table 1. CB1R and CB2R Affinity Values**

| SAMPLE | $IC_{50}$ on $CB_1$ nM (m ± sd, n=3) | Ki on $CB_1$ nM (m ± sd, n=3) | $IC_{50}$ on $CB_2$ nM (m ± sd, n=3) | Ki on $CB_2$ nM (m ± sd, n=3) |
|---|---|---|---|---|
| **$\Delta^9$-THCV** | 13.1 ± 4.1 | 1.5 ± 0.5 | 17.6 ± 3.9 | 2.8 ± 0.6 |
| **$\Delta^9$-tetrahydrocannabivarin-11-oic acid (5)** | 1681.5 ± 164.5 | 186.8 ± 78.3 | 3715.0 ± 287.1 | 589.7 ± 45.6 |
| **11-hydroxy-$\Delta^9$-tetrahydrocannabivarin (4)** | 22.7 ± 6.7 | 2.5 ± 0.5 | 29.8 ± 8.3 | 4.7 ± 1.3 |
| **8α-hydroxy-$\Delta^9$-tetrahydrocannabivarin (2)** | 230.1 ± 23.3 | 25.6 ± 2.6 | 807.7 ± 17.5 | 128.2 ± 2.8 |
| **8β-hydroxy-$\Delta^9$-tetrahydrocannabivarin (3)** | >10000 | >10000 | >10000 | >10000 |

Data represent mean values for three separate experiments performed in duplicate and are expressed as $IC_{50}$ (the concentration of competing ligand which displaces 50% of the specific binding of the radioligand) and $K_i$ (the concentration of the competing ligand that will bind to half the binding sites at equilibrium in the absence of radioligand or other competitors).

FIGURES

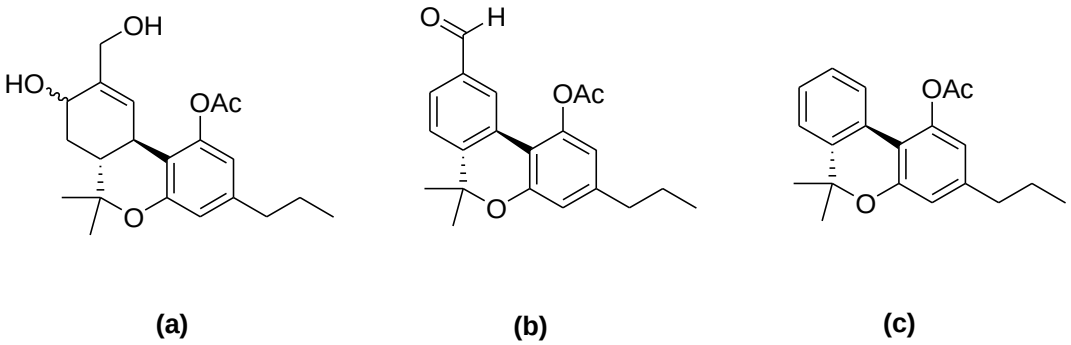
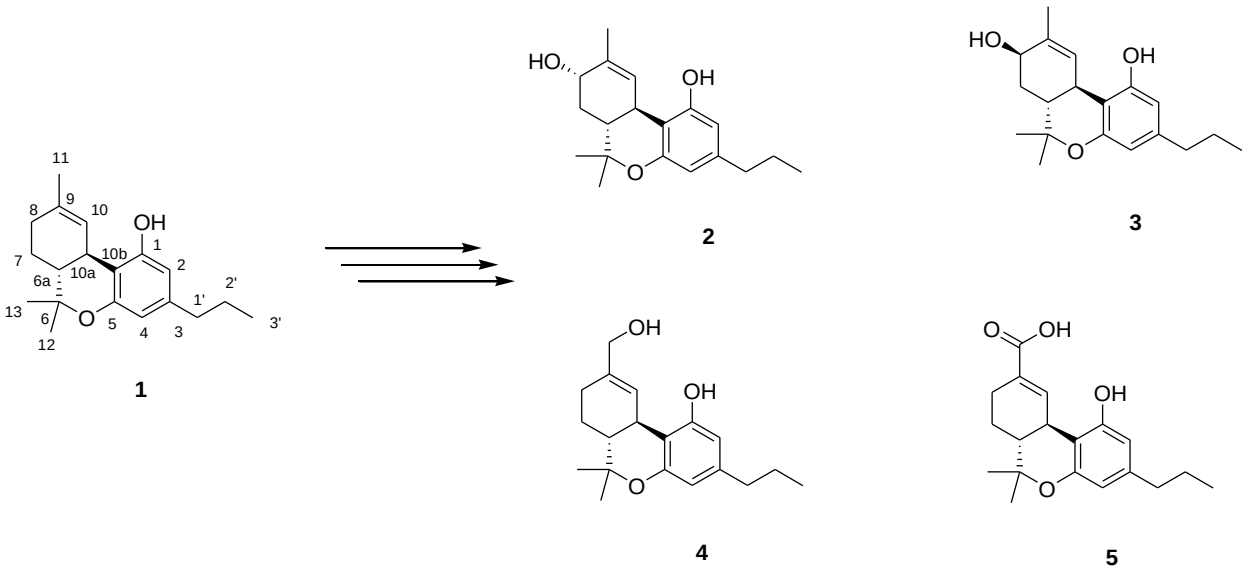
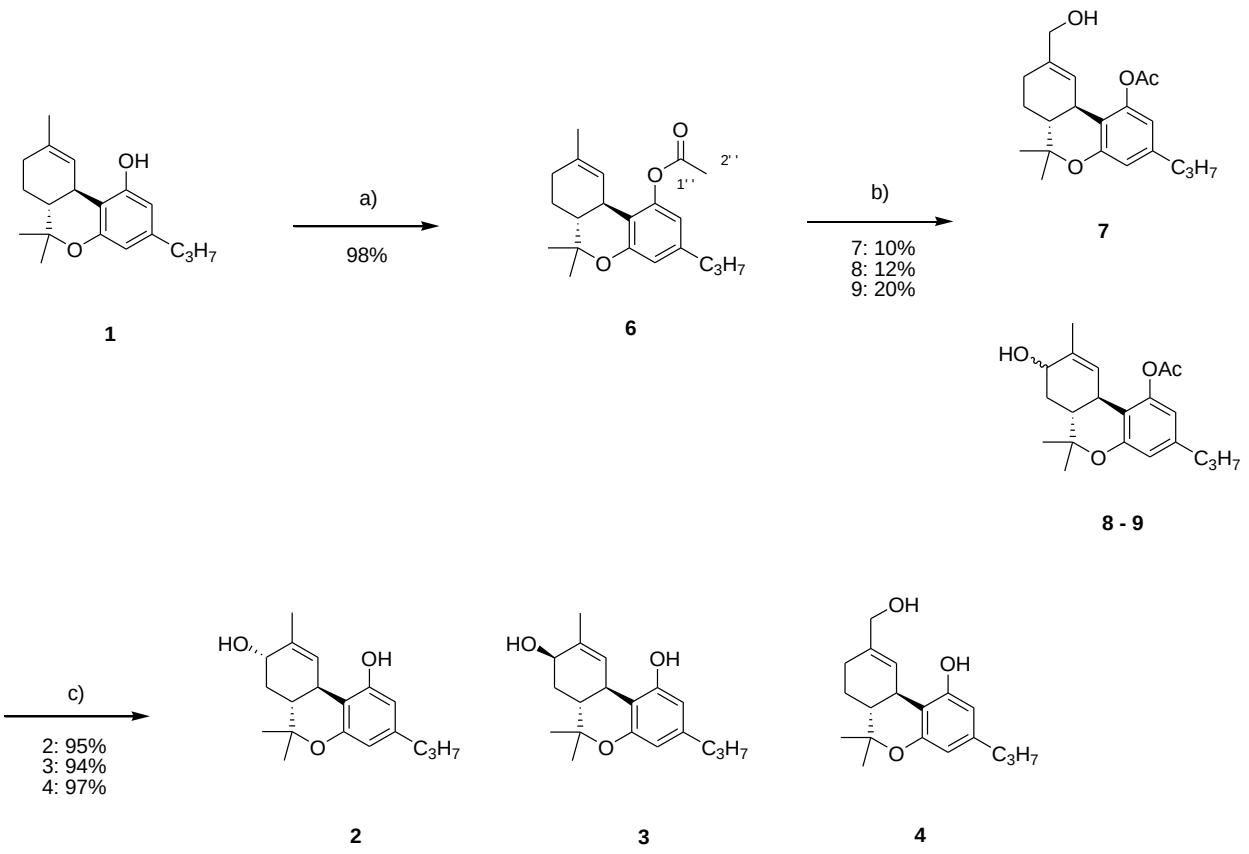



**Figure 1**. Examples of double oxidized derivatives and tricyclic dibenzopyrane byproducts.

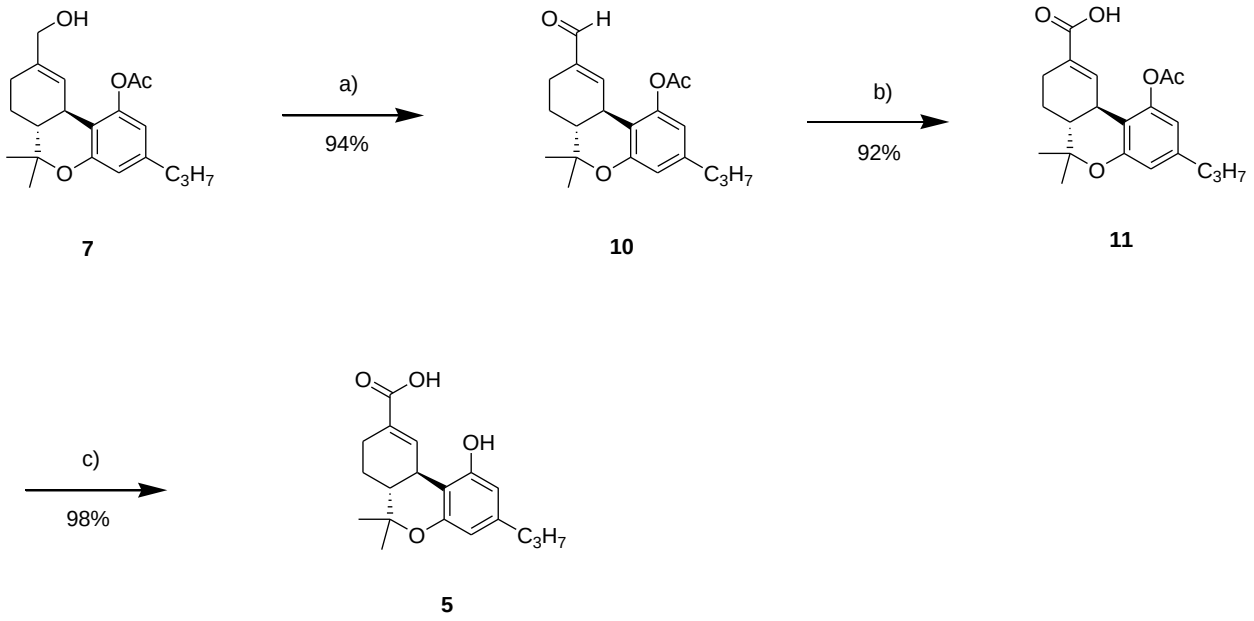
SCHEME




**Scheme 1**. THCV (**1**) and metabolites 8α-Hydroxy-$\Delta^9$-tetrahydrocannabivarin (**2**), 8β-Hydroxy-$\Delta^9$-tetrahydrocannabivarin (**3**), 11-Hydroxy-$\Delta^9$-tetrahydrocannabivarin (**4**), and $\Delta^9$-Tetrahydrocannabivarin-11-oic acid (**5**).




**Scheme 2**. Preparation of oxidized THCV metabolites (**2** – **4**). Reagents and conditions: (a) $Ac_2O$ in pyridine; (b) $SeO_2$ in EtOH at reflux; (c) NaOH aq.




**7** **10** **11** **5**

**Scheme 3**. Preparation of oxidized THCV metabolites (**5**). Reagents and conditions: (a) $MnO_2$ in $Et_2O$; (b) $NaClO_2$; (c) NaOH aq.

## ASSOCIATED CONTENT

**Supporting Information**.

$^1$H-NMR, COSY, HSQC edited spectra data of compounds **2** - **11**, and structure of the known compounds (PDF).

## AUTHOR INFORMATION

### Corresponding Author

* E-mail: francesco.tinto@icb.cnr.it

### Notes

The authors declare no competing financial interest.


## ACKNOWLEDGMENT

The authors thank to GW Research Ltd. for the supply of $\Delta^9$-THCV and for financial support. MK is recipient of a scholarship from INCIPIT PhD programme, which is co-funded by the COFUND scheme Marie Skłodowska-Curie Actions. We also thank Professor Vincenzo Di Marzo for his critical analysis of the data and review of this manuscript.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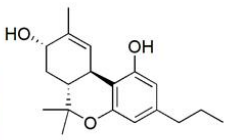
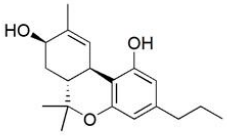



8$\alpha$-hydroxy-$\Delta^9$-tetrahydrocannabivarin

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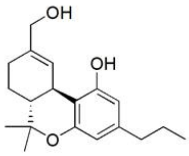


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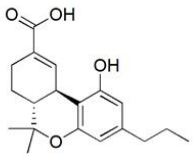


$\Delta^9$-tetrahydrocannabivarin-11-oic acid

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