

Mini-Review

11-Nor-9-carboxy- Δ^9 -tetrahydrocannabinol – a ubiquitous yet underresearched cannabinoid. A review of the literature

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Abstract

Synthetic Δ^9 -tetrahydrocannabinol (THC), pharmaceutical grade herbal cannabis as well as formulations of well-defined cannabis extracts are available as registered medicines in several countries. It is generally accepted that the main central and peripheral pharmacological effects of THC are mediated by CB1 and CB2 cannabinoid receptors. Cytochrome P450-mediated oxidations of THC afford the non-psychoactive 11-nor-9-carboxy-THC (THC-COOH) as main metabolite. The pharmacokinetics of THC-COOH has been studied thoroughly and this abundant acid metabolite has become an established urinary marker of cannabis consumption in forensic, clinical and environmental analyses. Surprisingly, however, data on the biological activity of this ubiquitous metabolite are scarce. A few studies have examined the effect of THC-COOH on the biosynthesis of prostaglandins and other eicosanoids, on capsaicin-sensitive sensory nerves, on the multidrug transporter P-glycoprotein, on the cannabinoid and estrogen receptors *in vitro*, as well as its anti-cataleptic, analgesic, platelet-activating factor inhibitory and anti-inflammatory activities *in vivo*; THC-COOH has also been reported to block certain behavioural effects of THC in rodents. Hereby we provide a literature review on the reported pharmacological effects of THC-COOH and advocate further studies to reveal any potential involvement of this abundant metabolite in the complex pharmacology and in the proven therapeutic effects of THC-containing preparations.

Keywords: cannabinoid, THC, metabolism, carboxylic acid, THC-COOH, pharmacology.

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Introduction

Δ^9 -Tetrahydrocannabinol (THC) (Figure 1) was identified five decades ago by Gaoni and Mechoulam [24] as the main psychoactive constituent of *Cannabis sativa*, preparations from which have been used for ritual, therapeutic as well as recreational purposes for millennia. Synthetic THC, pharmaceutical grade herbal cannabis as well as formulations of well-defined cannabis extracts are now available as registered medicines in several countries. Extensive studies on the pharmacodynamics and pharmacokinetics of THC have established that the central and peripheral effects of THC are

mainly mediated by the CB1 and CB2 receptors, but growing evidence indicates that other targets are also involved in the multifaceted pharmacology of THC and other cannabinoids. Since the early 1970s, the human metabolism of THC has also been clarified. Δ^9 -Tetrahydrocannabinol-11-oic acid (THC-11-oic acid or THC-COOH; Figure 2), being most abundant in bodily fluids, has become an established marker of cannabis consumption in forensic, clinical and environmental analyses. Unlike with the plant cannabinoids, however, the biological activity of this prominent metabolite has not been systematically investigated. Here the available information on the biological properties of THC-COOH with emphasis on human studies is reviewed.

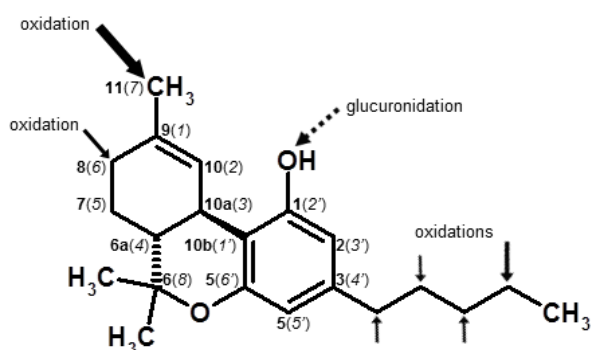


Figure 1. The chemical structure of THC showing both the dibenzopyran numbering (bold numerals) and the former terpene numbering (italic numerals in parenthesis). The main human metabolic transformations of THC are also shown. The arrow-size is proportional to the urinary abundance of the corresponding biotransformation product. The C-11 oxidation products may form esters and glucuronides that are not indicated (for details, see text).

Methodology

Exhaustive literature searches were carried out using textual and structural queries in electronic databases. Thus, structure-based queries for THC-COOH (with undefined stereochemistry) in SciFinder® (CAS, American Chemical Society) and Reaxys® (Elsevier) were completed by December 2013. Text searches for ‘tetrahydrocannabinol-7-oic acid’ (according to the monoterpene numbering used up to the mid-1980s) or ‘tetrahydrocannabinol-11-oic acid’ as keywords were done in PubMed (U.S. National Library of Medicine) for this period. Furthermore, the retrieved journal articles as well as scholarly books on marijuana and cannabinoids were scanned for possible additional publications. Data appearing only in patents were typically not used for the present overview.

Metabolism of THC

Following initial studies in animals in the 1970s observing considerable metabolic variations between species [2,32], the metabolism and disposition of THC in humans were also clarified and the subject has extensively been reviewed [1,2,30,31,33,37,54,76,77,82,85]. Figure 1 summarises the key metabolic pathways. The initial, Phase I metabolites arise from sequential allylic oxidations, as well as hydroxylation and β -oxidation of the pentyl side-chain of THC, and all appear to be catalysed by cytochrome P450 isoenzymes primarily in the liver [7,34,63,81]. Such metabolism was also found in brain cell cultures [53,80]. The initial oxidative metabolite of THC, i.e., 11-hydroxy-THC (11-OH-THC), is psychoactive. Further oxidation of 11-OH-THC leads, via the corresponding transient aldehyde [5,79], to the less lipophilic and non-psychoactive THC-COOH [17,75] (Figure 2). Some of the oxidized metabolites, including THC-COOH, are subsequently conjugated to form glucuronides as Phase II

metabolites [26,51,83]. In rodents, sulfate and fatty acid ester conjugates have also been detected [37,48]. THC-COOH is the most abundant metabolite excreted with urine and feces either as free acid or as glucuronide ester conjugate that is reflected by their presence in urban sewage and some surface waters at the tens of ng/l level [18,44,62].

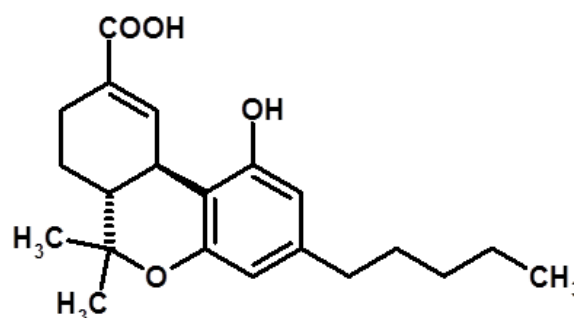


Figure 2. The structure of THC-COOH.

Molecular formula: $C_{21}H_{28}O_4$

Molecular weight: 344.4

Chemistry and physicochemical properties of THC-COOH

Names

IUPAC systematic name: (6*aR*,10*aR*)-1-hydroxy-6,6-dimethyl-3-pentyl-6*a*,7,8,10*a*-tetrahydro-6*H*-benzo[*c*]chromene-9-carboxylic acid

Chemical Abstract Service name (prior to 1972): (6*aR*,10*aR*)-6*a*,7,8,10*a*-tetrahydro-1-hydroxy-6,6-dimethyl-3-pentyl-6*H*-dibenzo[*b,d*]pyran-9-carboxylic acid

Other names: (-)-11-nor- Δ^9 -tetrahydrocannabinol-9-carboxylic acid; (-)-11-nor-9-carboxy- Δ^9 -tetrahydrocannabinol

Physicochemical properties

White solid, melting point: 205–207 °C [61]; 216–218 °C [55]

Optical rotation: $[\alpha]_D^{27}$ -160 (c 0.00515, EtOH) [66]; $[\alpha]_D^{25}$ -164 (c 0.15, $CHCl_3$) [41]

The amphiphilic metabolite is endowed with a polar headgroup (-COOH), a rigid hydroxydibenzopyran core capable of forming H-bonds, and a flexible lipophilic side chain. LogP: 2.24 (at pH=7.4) [68] (for THC, logP values of 3.8 [25]; and 4.1 [72] have been reported). Polar surface area: 70 Å² (calculated by Maestro 9.4 program, Schrödinger, New York). The polar surface area measures the transport properties of a drug through biomembranes. Drugs acting on the central nervous system typically have a polar surface area below 70 Å²; orally administered substances with a polar surface area larger than 120 Å² do not cross the blood-brain barrier by passive transport [43,74].

Human pharmacokinetics of THC and its metabolites

Depending on the route and frequency of administration of THC-containing preparations, THC-COOH is present in blood plasma typically in the 3–150 ng/ml (0.01–0.44 nM) concentration range. THC-COOH begins to appear in plasma about 30 min after dosing, its plasma level peaks between 60–180 min and remains elevated (>20 ng/ml) for 10–20 h either upon oral or intravenous administration or smoking of THC preparations [38,47,76]. In general, the plasma concentration of free THC-COOH is lower than the concentration of its glucuronide conjugate, and the latter is also more abundant in urine and feces [47,69,78]. An early human study, however, noted that intravenously injected THC-COOH (20 mg) disappeared slowly (>24 h) from the blood and no sign of further oxidative metabolism was found [76]. Genetic polymorphism in CYP450 enzymes may influence the oxidative metabolism of THC and, consequently, blood THC-COOH levels [63]. In a recent study, after seven days of continuous oral administration of THC (2–7 daily oral doses of 20 mg in Marinol® capsules), the mean plasma concentration of THC-COOH was found to be 327 ng/ml with about an equal amount of its glucuronide being also present [65]. Upon sprayed oromucosal applications of 5.4 and 16 mg of THC (in a 1:1 blend of THC and cannabidiol (CBD); Sativex®), the respective THC-COOH plasma concentration ranges were about 45–105 and 70–120 ng/ml during the 2–6 h post-dose period [42]. A human pharmacokinetics study with THC-COOH (5 mg, i.v. over 10 min) indicated mean peak serum concentration of 337 ng/ml followed by a rapid decline to below 50 ng/ml after 5 h; the complete distribution of the drug lasted 12 h and the mean elimination half-life was relatively short (~17 h) [27].

Studies involving 'passive' consumption of THC

When late-term pregnant rhesus monkeys were given THC (0.3 mg/kg, i.v.), negligible transfer of THC-COOH from maternal plasma (44 ng/ml peak concentration) to fetal plasma (<2.0 ng/ml) was detected and no acid metabolite was found in the placenta or other fetal tissues analysed [4]. Analysis for drugs of the placenta of women (n = 64) who voluntarily interrupted their pregnancy in the first trimester of pregnancy indicated in one case the presence of THC-COOH at 123 ng/g [40]. Analysis of the milk of a breastfeeding marijuana-smoking mother indicated 60, 1.1 and 1.6 ng/ml of THC, 11-OH-THC and THC-COOH, respectively, while the baby's whole fecal sample contained 347, 67 and 611 ng of THC, 11-OH-THC and THC-COOH, respectively [60]. An unusual study noted that ingestion of THC-COOH via the milk (51 ng/ml mean acid level) from hemp-grazing buffaloes the metabolite was detectable at low level (8 and 27 ng/ml) in the urine of some children regularly drink-

ing such milk [3]. In a study assessing the placental transfer of cannabinoids of marijuana user pregnant women, the concentration of THC-COOH in umbilical cord blood at delivery ranged from 0.4 to 18 ng/ml, which was 4 to 7 times lower than in maternal blood [6]. A recent analysis of 16 umbilical cord segments detected THC-COOH in seven samples (concentration range: 0.07–6.1 ng/g) [19]. In the first feces of neonates of cannabis-using pregnant women, the total THC-COOH content of the meconium ranged from 5 to 250 ng/g [21,28,58]. In a case-series of autopsies with initial cannabinoid-positive drug test (n = 30), the mean THC-COOH concentrations of cardiac and peripheral blood were 57 and 61 ng/ml, respectively, while the corresponding values for THC were 8.0 and 16 ng/ml [49]. Analyses of fluids and tissues of post mortem cases showed that THC-COOH was particularly abundant in bile (up to 1500 ng/g) [22,29].

Interaction with other drugs

Inhibitors of CYP450 enzymes, such as barbiturates, ketoconazole, rifampicin, sulfaphenazole, SKF525A and CBD may hinder the oxidative metabolism of THC, thus the formation of THC-COOH, in humans [7,25,34,35,67,70,81]. The induction of CYP450-dependent alcohol oxygenases by certain steroids, however, might lead to elevated levels of THC-metabolites in vivo [23].

Biological activity studies

Compared to THC, relatively few published studies have examined the pharmacology of THC-COOH either in vitro or in vivo. What follows is an enumeration of publications of in vitro studies as well as animal and human experiments. (It may be noted that practically nothing is known about the biological activity of the glucuronide conjugates.)

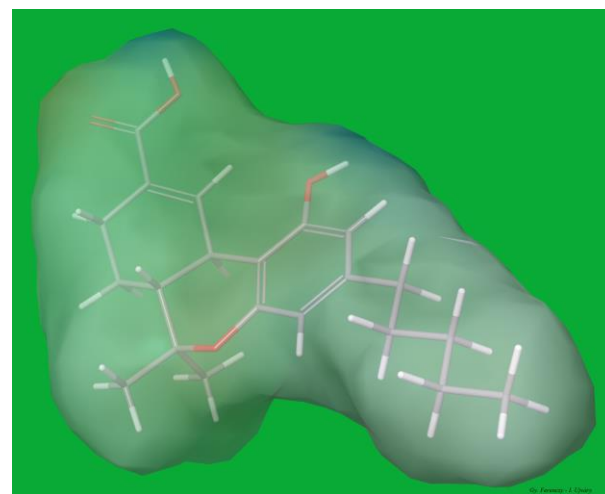


Figure 3. Picture of the molecular model of THC-COOH with its electrostatic potential surface.

Δ^8 -THC-COOH and ajulemic acid - a cautionary remark

Speculating that some of the conflicting effects of THC observed *in vivo* are due to metabolites, Burstein initiated studies with THC-COOH and its analogues [10]. These investigations have culminated in the development of ajulemic acid, which is a homologous Δ^8 -isomer of THC-COOH, as an analgesic and anti-inflammatory cannabinoid [9]. Several other metabolic studies have also used the more readily available Δ^8 -THC (see, for example, [86]). However, the structural changes (double bond isomerisation and homologisation) appear to affect bioactivity substantially thus the plethora of biological activity of data accumulated for the synthetic Δ^8 -THC-COOH and ajulemic acid are not applicable to the genuine THC-metabolite, i.e., THC-COOH, and should be interpreted with prudence.

Receptor studies

An early study with a series of cannabinoids Nye et al [56] examined the inhibition of the binding of a water-soluble THC-analogue to rat neuronal membranes and the inhibition of electrically evoked contractions of guinea-pig ileum preparations. For the membrane, THC-COOH had low affinity ($K_i = 452$ nM), while for the ileum preparation it was inactive ($IC_{50} > 10,000$ nM); in the case of THC, the respective K_i and IC_{50} values were 27 and 100 nM. In a later study with purified rat brain cortical receptor, THC-COOH showed no activity ($K_i > 10,000$ nM) [20].

Studies *in vitro* and in animals

Unlike THC, the acid metabolite (at 3 μ M) did not activate capsaicin-sensitive sensory nerves as demonstrated by its failure to produce vasodilation in rat artery preparations [88]. THC-COOH (at 20 μ M) failed to compete with estradiol in a rat uterine estrogen receptor preparation [64]. Among the cannabinoids tested as potential inhibitors of the drug efflux transporter P-glycoprotein (Pgp), which is responsible for the multi-drug-resistance of tumour and normal cells, THC-COOH behaved as a substrate and was the most active in stimulating Pgp-dependent ATPase (1.3-fold increase); CBD was an inhibitor [87]. The acid also failed to inhibit another multidrug transporter protein type [36].

A series of laboratory experiments by the Burstein-group [10] revealed a complex pharmacokinetic-pharmacodynamic relationship between THC and its acid metabolite due to their interference with the eicosanoid pathway. In an early study, THC-COOH was not analgesic in the mouse (hot plate test, max. dose 50 mg/kg s.c.) [84], but in a different study it displayed analgesic and anti-inflammatory properties apparently by inhibiting cyclooxygenase and 5-lipoxygenase activities, i.e., the production of pro-inflammatory eicosanoids (e.g., prostaglandins) from arachidonic acid (AA) in human lung fibroblast or mouse brain preparations or by purified enzymes [11,14]. This effect thus counteracts the THC-stimulated release of AA. The effect of THC-COOH on AA release from membranes appears

to depend on the cell type and the assay conditions: in intact neuroblastoma cells, the metabolite is a meagre stimulator of arachidonic acid release than THC [16], while in brain synaptosomal preparation, the drug-induced stimulation of phospholipases A₂ and C lead to significant increases in AA levels [39]. THC-COOH also inhibited 15-lipoxygenase at sub-micromolar level *in vitro* indicating anti-atherosclerotic potential *in vivo* [71].

In the mouse, oral THC-COOH was slightly more active than THC in preventing platelet factor-induced edema and mortality indicating again its potential involvement in the anti-inflammatory and anti-asthmatic action of the phytocannabinoid [12]. THC-COOH (10 mg/kg, oral) exhibited analgesic activity in the mouse (hot plate test) with an earlier onset as compared to either enantiomer of THC indicating the involvement of the metabolite in the anti-nociceptive effects of THC [13]. Furthermore, unlike aspirin or indomethacin, THC-COOH (50 mg/kg) was not ulcerogenic when administered intra-gastrically to rats [8]. Interestingly, not only THC-COOH but also its enantiomer had topical anti-inflammatory effects in the chemically-(PMA)-induced ear edema assay in the mouse [73].

Upon oral administration to mice, THC-COOH was not cataleptic [13], but prevented THC-induced catalepsy [15]. Since only a negligible fraction (0.2%) of injected THC-COOH reached the brain, peripheral mode of action for this anti-cataleptic effect was suggested. In the mouse tetrad assay, THC-COOH was inactive ($ED_{50} > 52$ mg/kg, i.v.); in the rat, however, sustained THC-like immobility was induced at the 2.8 mg/kg dose upon i.p. injection [50].

In the elevated plus-maze test with mice, THC-COOH at doses up to 20 mg/kg (i.p.) was not anxiolytic or anxiogenic but abolished the anxiogenic behavioural effect of THC [57]. The diazepam-like behavioural effects of both nabilone and CBD displayed in this assay system were blocked by THC-COOH. The acid, however, failed to prevent the cataleptic effects of THC in this study. The isomeric Δ^8 -THC-COOH displayed no overt behavioural activity in the rhesus monkey [52].

In a rat pineal gland preparation, THC-COOH as well as THC, CBD and cannabinol attenuated melatonin biosynthesis by reducing norepinephrine-regulated aralkylamine *N*-acetyltransferase activity apparently by a CB-receptor-independent way [45]. In excitotoxically lesioned NMDA-receptor-rich dentate gyrus culture from rat brain, THC-COOH reduced the number of microglial cells but, unlike 2-AG, was not neuroprotective [46].

Human studies

Human (psycho)pharmacology studies with THC-COOH are scarce. Similar to the Δ^8 -THC-COOH isomer in monkeys (see above), THC-COOH (20 mg administered by i.v. over 8 min) failed to elicit any observable physiological effects, including changes in heart rate or feeling of marijuana-like "high" in hu-

mans [59]. In an already mentioned study on the pharmacokinetics of THC-COOH (5 mg, i.v.) [27], no changes in ECG parameters were noted, but two of the 10 participants experienced adverse effects: one reported transient headache and nausea 7 h after drug administration, while the other reported sweating at night two days after the experiment. While demonstrating that THC-COOH lacks psychoactivity, none of these studies, however, were designed to reveal any therapeutically useful – or, for that matter, harmful – properties of the metabolite.

Summary

Review of the literature on THC-COOH indicates that the pharmacological properties of this non-psychoactive but prominent metabolite have been incompletely and – in the authors' opinion – insufficiently characterized. The main unanswered questions are: Could any of the pharmacological effects observed for THC be attributed to THC-COOH? To what extent THC-COOH affects the biological activity of THC-containing preparations? It is hoped that further studies will reveal any potential involvement of this abundant and long-lived metabolite in the complex pharmacology and in the proven therapeutic effects of THC-containing medicines. In other words: could THC be a potential pro-drug to another pharmacological entity?

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Previous publication

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