CHAPTER 2

Biotransformation of Cannabinoids

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Abstract

Cannabinoids are terpenophenolic compounds consisting of an aromatic polyketide and derived from the geranyl diphosphate C_{10} terpenoid unit. They are the active constituents in *Cannabis sativa* and have been utilized in a number of cannabis-based medicines. Biotransformation of cannabinoids is an important field of xenobiochemistry and toxicology and the study of the metabolism of these compounds can lead to the discovery of new compounds, unknown metabolites with unique structures and new therapeutic entities. Different fungi, bacteria, plants and animal cells have been used for the regio- and stereoselective transformation of cannabinoids. All of the above mentioned organisms have distinct enzymes which catalyze the conversion of a specific cannabinoid at different positions and thus provide a variety of derivatives. All organisms are able to transform the alkyl side chain where as mammalians are unique in the formation of the carboxy derivatives. This review article assesses the current knowledge on the biotransformation of Δ^8-THC, CBN, CBD with particular focus on Δ^9-THC.

Introduction

Pharmacological properties of cannabis are well known and documented in literature (Carter and Weydt 2002; Russo 2007). The term "cannabinoids" represents a group of C_1, C_3 and C_5 side chains terpenophenolic compounds found until now only in *Cannabis sativa L* (Cannabaceae) (Mechoulam and Goani 1967). Cannabinoids, the active compounds in *C. sativa* (Sirikantaramas et al. 2007) have distinct therapeutic properties. After the purification and structure elucidation, the best known cannabinoids, Δ^9-Tetrahydrocannabinol (Δ^9-THC) (Gaoni and Mechoulam 1964), Cannabidiol (CBD), Cannabiniol (CBN), and Δ^8-Tetrahydrocannabinol (Δ^8-THC) (Fig. 1) were extensively studied and found to possess a variety of potentially useful pharmacological

activities in addition to the psychotropic effects for which cannabis is well known (Monique and Stephen 1976). Such physiological actions as anticonvulsant, anti-emetic, anti-oxidative, neuroprotective, anti-inflammatory, antidepressant, hypotensive, bronchodilation and lowering of intraocular pressure have led to a number of investigations on the possible development of useful medicines from the naturally occurring and synthetic cannabinoids. THC is the psychoactive component of the cannabis plant (Scotter et al. 2010), while the major non psychoactive constituents include CBD, CBN, cannabigerol (CBG) and cannabichromene (CBC) (Fig. 1) (Gaoni and Mechoulam 1966). In the plant these compounds occur in their respective acidic form. i.e. having a carboxylic acid group. The acidic cannabinoids, which are non psychotropic, undergo decarboxylation upon heat treatment (e.g. smoking) to yield the physcotropic cannabinoids. Δ^9-THC has been used to treat number of disease states (e.g. pain, anxiety, asthma, glaucoma, hypertension) but also possesses some undesirable pharmacological side effects (e.g. euphoria, tachycardia, etc.) (Abbott et al. 1977). To minimize these undesirable side effects new cannabinoids have been prepared by de novo synthesis (Pars and Howes 1977) and by chemical and microbiological conversions (Abbott et al. 1977; Binder and Meisenberg 1978; Christie et al. 1978; Robertson et al. 1978b) of both naturally occurring and synthetic cannabinoids. This review assesses the available information regarding the production of various compounds obtained from the biotransformation of cannabinoids by using different microorganisms, plant cells and mammalian cells. In this review the benzoypyran numbering system is used instead of the monoterpene numbering system (under the monoterpene system Δ^9-THC is known as Δ^1-THC) (Fig. 2).

Biotransformation

Biotransformation can be defined as the enzymatic conversion of natural and

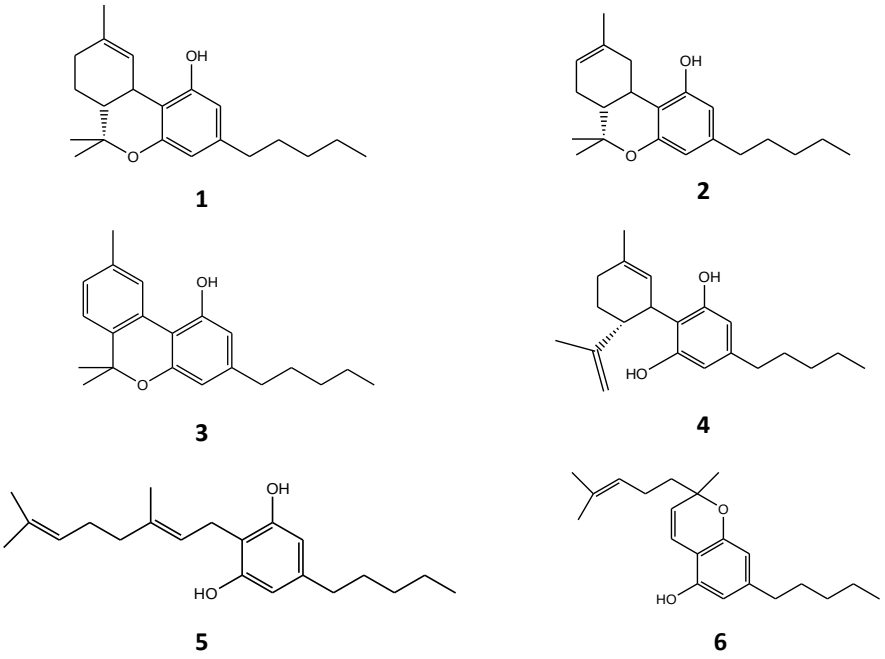


Figure. 1: Chemical structures of the major cannabinoids. **1**: Δ^9-THC, **2**: Δ^8-THC, **3**: CBD, **4**: CBN, **5**: CBG, **6**: CBC.

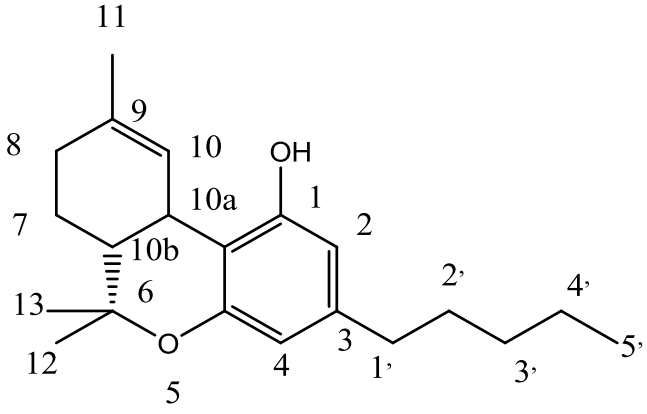


Figure 2: Δ^9-THC (Benzopyran numbering system)

synthetic compounds into substances having specifically modified structures (Venisetty and Ciddi 2003). Biotransformation is a vital process for survival, in that it transforms absorbed nutrients into substances required for normal bodily functions and to detoxify xenobiotics. These reactions convert non polar compounds into more polar compounds, making them water soluble and more easily excretable for the organism (Asha and Vidyavathi 2009). In this way biotransformation serves as an important defense mechanism in that, toxic xenobiotics and body wastes are converted into less harmful substances which can be easily excreted from the body. The pathways of xenobiotic metabolism can be divided into phase I and phase II reactions. Phase I includes oxidative, reductive and hydrolytic reactions. In these reactions, a polar group is introduced, so the molecule becomes more polar and thus improves excretion from the cell/or body. In phase II, polar endogenous molecules like glucoronic acid, sulfate, glycine, glutathione etc. are attached to form highly water soluble substances. These reactions are known as conjugation reactions (Smith and Rosazza 1983).

Active metabolites obtained from biotransformation

Plant, animal, microbial cells and purified enzymes are being used to carry out specific conversions of complex substances. This approach has great potential to generate novel products (Giri et al. 2001). Biotransformation reactions yield chemically stable metabolites which can be pharmacologically active (Baillie et al. 2002, Fura et al. 2004, Garattini 1985, Guengerich 2001, Kumar and Surapaneni 2001) and might thus significantly contribute to the therapeutic effect of a drug. Some of these have even been developed as drugs in their own right. Important examples are atorvastatin (Lennernas 2003; Williams and Feely 2002), simvastatin (Thomayant et al. 2003; Williams and Feely 2002),

fluoxetine (Cheer and Goa 2001), cetirizine (Golightly and Greos 2005) and fexofenadine (Golightly and Greos 2005, Meeves and Appajosyula 2003).

In a traditional prodrug-based approach to drug discovery, biotransformation reactions can play a key role to convert pharmacologically inactive compounds to active metabolites with strong therapeutic effects through phase I (oxidative or reductive) and phase II (conjugative) metabolism. Some of the important biotransformation reactions are aliphatic or aromatic carbon hydroxylation, epoxidation, heteroatom oxidation (N, S, and P), reduction, glucuronidation, sulfation and acetylation.

Microorganisms in biotransformation

The biotransformation potential of microorganisms and their enzymes for the production of a large library of novel metabolites is well documented (Nikolova and Ward 1993, Schulze and Wubbolts 1999, Ward and Singh 2000). The use of microbial cell cultures for transformation of natural products is favorable because they mimic mammalian biotransformation (Smith and Rosazza 1983). Microbial models offer a number of advantages over the use of animals in metabolism studies, such as a reduction in the number of animals used (particularly in the early phases of drug development), ease of setup and manipulation (microbial models can be scaled up easily for the preparation of large quantities of metabolites) and cost effectiveness. In addition, methods for genetic modification of microbes are well established (Rathbone and Bruce 2002). Microbial strains with specific properties for certain chemical conversions may be selected or even engineered for stereoselective and regiospecific conversions (Abourashed et al. 1999, Demain 2000, Rathbone and Bruce 2002, Smith and Rosazza 1983). The maintenance of stock cultures of microorganisms is also easier and cheaper than the maintenance of mammalian cell and tissue cultures or laboratory animals. Microorganisms can also be

utilized to perform reactions which are difficult to perform chemically (Venisetty and Ciddi 2003).

Plants and plant cells in biotransformation

Plants are able to produce a number of diverse biochemicals including drugs, flavors, pigments and agrochemicals which cannot easily be achieved by synthetic means. Plant cell cultures have great ability to produce specific secondary metabolites like a wide variety of chemical compounds including aromatics, steroids, alkaloids, coumarins and terpenoids. Production of secondary metabolites and the biotransformation of precursor compounds by plant tissue cultures may lead to both the synthesis of valuable substances and elucidation of their biosynthetic pathways (Berlin et al. 1989, Loh et al. 1983). Production and accumulation of plant specific secondary metabolites are not always possible in plant cell cultures. However such cultures may retain an ability to transform exogenous substrates into products of interest. Plant enzymes act as suitable biocatalysts to perform complex biochemical reactions (Giri et al. 2001). The chemical compounds, which can undergo biotransformation by plant enzymes, are diverse in nature (Franssen and Walton 1999) and are not necessarily natural intermediates in plant metabolism but can also be of synthetic origin (Pras et al. 1995). Plant cell cultures and enzymes have thus the potential to transform substances, such as industrial byproducts, into novel, valuable products. Plant biotransformation systems can be used alone or in combination with organic synthesis to produce novel chemicals.

Biotransformation of cannabinoids

Biotransformation of cannabinoids by mammalian cells

Extensive *in vivo* and *in vitro* studies have been carried out on the biotransformation of cannabinoids by mammalian cell cultures.

Tetrahydrocannabinol (THC) and other cannabinoids are shown to be good substrates for cytochrome P450 (CYP450) enzymes because of their hydrophobic properties (Yamamoto et al. 1995). Mammalian CYP450s metabolise Δ^9-THC into a number of new compounds. Many oxidative metabolites of tetrahydrocannabinols (THCs) such as 11-hydroxy-THC, 11-oxo-Δ^8-THC, 7-oxo-Δ^8-THC, 8β,9β-epoxyhexahydrocannabinol (EHHC), 9α,10α-EHHC and 3'-hydroxy-Δ^9-THC (Table 3) are pharmacologically more active than THC (Yamamoto et al. 2003).

Metabolism of THC in humans

The metabolism of Δ^9-THC and related cannabinoids in human has been studied by utilizing parenteral, oral, and smoking as a route of administration. The general pattern of metabolism is the same in all cases with as major metabolic reactions the formation of an 11-hydroxy derivative and as minor product the 8-hydroxy derivative (Wall and Perez-Reyes 1981). The monocarboxylated derivatives have been isolated from human urine (Table 3, compound 20 and 23), further side chain carboxylated and their hydroxylated derivatives occur and were also found in urine (Table 4, compound 27, 28, 29, 30, 31, 32, 34, 35, 36, 37, 39) (Halldin et al. 1982a, Halldin et al. 1982b). Among the acid derivatives, Δ^9-THC-11-oic acid (Table 3, compound 20) (Halldin et al. 1982b, Kanter and Hollister 1978), 4',5'-bisnor-Δ^9-THC-11,3'-dioic acid (Table 4, compound 35) (Halldin et al. 1982a) and Δ^9-THC-11-oic acid glucoronide (Fig. 3a) (Williams and Moffat 1980) are identified as the major metabolites in human urine. *In vitro* human liver metabolism yields 8α-hydroxy- (Table 1, compound 2) (Bornheim et al. 1992, Widman et al. 1979), 11-hydroxy-, 8β-hydroxy- (Table 1, compound 1, 3) (Bornheim et al. 1992, Halldin et al. 1982c, Wall et al. 1972, Widman et al. 1979), traces of 11-hydroxy-

epoxyhexahydrocannabinol (11-OH-EHHC) (Fig. 3b) (Halldin et al. 1982c) and 8-keto-Δ^9-THC (Fig. 3c) (Bornheim et al. 1992).

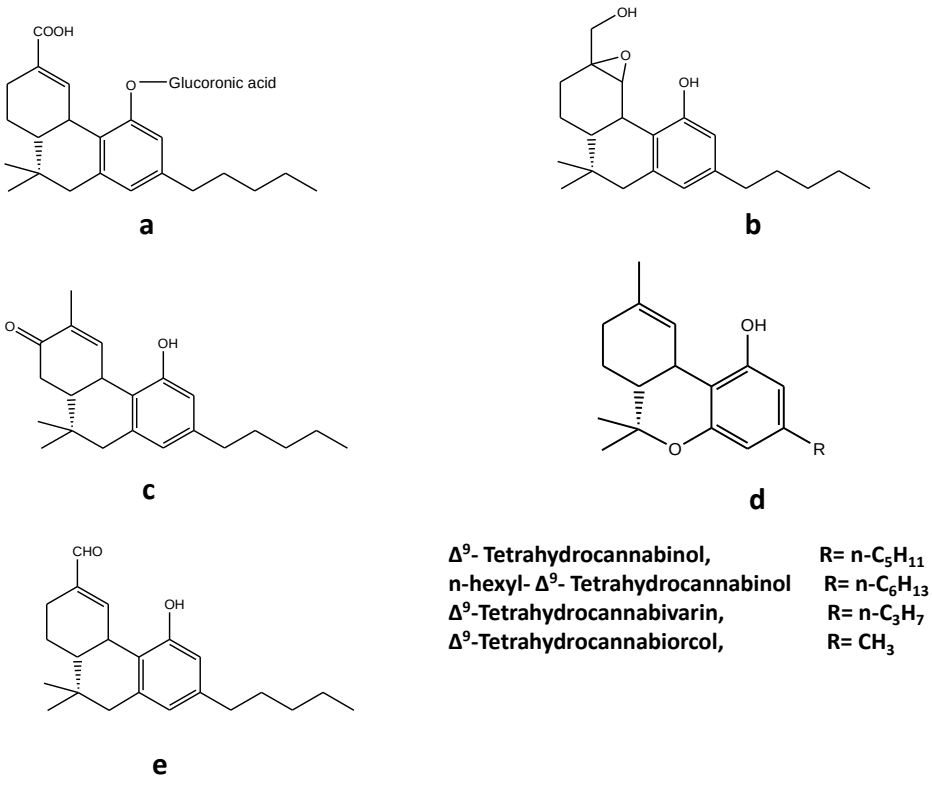


Figure 3: a; Δ^9-THC-11-oic acid glucoronide **b**; 11-hydroxy-EHHC **c**; 8-keto-Δ^9-THC **d**; Derivatives of THC **e**; 11-oxo- Δ^9- THC.

Metabolism of THC in rabbits

After *in vivo* administration of the Δ^9-THC to rabbits, a number of mono- and dihydroxy derivatives (Table 1 ,2) (Nordqvist et al. 1979a), monocarboxylic acid (Table 3, Compound 20) and side chain acids (Table 4, compound 26, 27, 29, 31, 32, 33, 34, 35, 38, 39) (Burstein et al. 1972, Nordqvist et al. 1979a) were found in the rabbit urine including the 4',5'-bisnor-Δ^9-THC-11,3'-dioic acid

(Table 4, compound 35) as a major compound (Nordqvist et al. 1974). Furthermore, three side-chain monocarboxylic acids hydroxylated in the allylic position in the isoprene moiety were also identified as O-glucuronide in rabbit urine (Nordqvist et al. 1979b). Rabbit *in vitro* liver microsomal metabolism of Δ^9-THC formed 11-hydroxy- (Table 1, compound 1) (Burstein and Kupfer 1971a, Nilsson et al. 1970; Wall et al. 1970) and 8α-hydroxy-Δ^9-THC (Table 1, compound 2) (Benzvi and Burstein 1975). Microsomal oxygenase was found to catalyze the oxidation of 11-hydroxy-Δ^8-THC to 11-oxo-Δ^8-THC (Watanabe et al. 1979).

Table 1. Monohydroxylated metabolites of Δ^9- THC. (1) man, (2) rhesus monkey**,** (3) mouse, (4) rat, (5) rabbit, (6) guinea pig, (7) dog, (8) microorganisms.

		Species								References
No	Position of OH	1	2	3	4	5	6	7	8	
1	11-OH	+	+	+	+	+	+	+	+	(Burstein and Kupfer 1971a, Burstein and Kupfer 1971b, Matsunaga et al. 1995, Nilsson et al. 1970, Wall et al. 1970)
2	8α-OH	+	+	+	+	+	+	+	+	(BenZvi et al. 1974, Harvey and Paton 1976, Jones et al. 1974, Matsunaga et al. 1995, Wall et al. 1972, Widman et al. 1975a)
3	8β-OH	+	+	+	+	+	+	+	+	(Harvey et al. 1977, Matsunaga et al. 1995, Wall 1971, Wall et al. 1972, Widman et al. 1975a)
4	1'-OH	−	−	−	−	−	+	−	+	(Binder and Popp 1980, Harvey et al. 1978b, Harvey et al. 1980a)
5	2'-OH	+	−	−	−	−	+	−	+	(Binder and Popp 1980, Halldin et al. 1982c, Harvey et al. 1978b, Harvey et al. 1980a)
6	3'-OH	−	−	+	+	+	+	+	+	(Binder 1976, Christie et al. 1978, Harvey et al. 1977, Harvey et al. 1978b, Harvey et al. 1980a, Widman et al. 1975a)
7	4'-OH	−	−	+	−	−	+	+	+	(Binder 1976, Binder and Meisenberg 1978, Binder and Popp 1980, Harvey and Paton 1978, Harvey et al. 1980a, Robertson and Lyle 1975, Widman et al. 1975a)

Metabolism of THC in mouse

Harvey and Paton (1978) identified multiple substituted metabolites formed by *in vivo* metabolism of Δ^9-THC in the mouse liver among which, 11-; 2'-; and 3'-

Table 2. Di- and trihydroxy metabolites of Δ^9-THC. (1) man, (2) rhesus monkey, (3) mouse, (4) rat, (5) rabbit, (6) guinea pig, (7) dog, (8) microorganisms.

		Species								References
No	Position of OH Groups	1	2	3	4	5	6	7	8	
9	8α , 11-di-OH	+	+	+	+	+	+	–	–	(Benzvi Z. and Burstein 1975, Halldin et al. 1982c, Harvey and Paton 1976, Harvey et al. 1978b, Harvey et al. 1980a, Jones et al. 1974, Wall and Brine 1976, Wall et al. 1972)
10	8β, 11-di-OH	+	+	+	+	–	+	+	–	(Halldin et al. 1982c, Harvey et al. 1977, Harvey et al. 1978b, Harvey et al. 1980a, Wall and Brine 1976)
11	1', 11-di-OH	–	+	–	–	–	+	–	–	(Harvey et al. 1980a, Wall and Brine 1976)
12	2', 11-di-OH	+	+	+	–	–	+	–	–	(Halldin et al. 1982c, Harvey et al. 1977, Harvey et al. 1978b, Harvey et al. 1980a, Wall and Brine 1976)
13	3', 11-di-OH	–	+	+	+	–	+	–	+	(Christie et al. 1978, Halldin et al. 1982c, Harvey et al. 1977, Harvey et al. 1978b, Wall and Brine 1976)
14	4', 11-di-OH	+	+	+	–	–	+	–	+	(Binder 1976, Halldin et al. 1982c, Harvey and Paton 1978, Harvey et al. 1980a, Wall and Brine 1976)
15	4', 8α-di-OH	–	–	–	–	–	–	–	+	(Binder 1976)
16	1'-4', 8β-di-OH	–	–	–	–	–	+	–	–	(Harvey et al. 1980a)
17	1', 2'-4'-di-OH	–	–	–	–	–	+	–	–	(Harvey et al. 1980a)
18	2'-4',8α,11-tri- OH	–	–	+	–	–	+	–	–	(Harvey and Paton 1978, Harvey et al. 1977, Harvey et al. 1978b, Harvey et al. 1980a)
19	2'-4', 8β,11-tri- OH	–	–	–	–	–	+	–	–	(Harvey et al. 1980a)

the monohydroxy derivatives (Table 1, compound 1, 5, 6), 8β, 11-; 2', 11-; and 3', 11-dihydroxy derivatives (Table 2, compound 10, 12, 13), 2', 8α, 11- and 3', 8α, 11- the most abundant trihydroxy derivatives (Table 2, compound 18). Harvey and Paton (1978) also found the formation of 2',11-, and 3',11-dihydroxy-8-oxo-Δ^9-THC (Table 6, compound 48) in mouse liver and 4'-hydroxylation (Table 1, compound 7) was suggested as a major metabolic route for the Δ^9-THC transformation (Harvey et al. 1977). Mono- and dihydroxy-acids of Δ^9-THC have also been found in mouse liver. Δ^9-THC-11-oic acid

(Table 3, compound 20) was identified from the mouse liver together with its 8α- and 2'- monohydroxy derivative (Table 5, compound 41 and 43), 2',8α- and 3',8α-dihydroxy derivatives (Table 5, compound 45) (Harvey and Paton 1976; Harvey et al. 1978b). *In vitro* mouse liver microsomes transformed Δ^9-THC into 11-hydroxy- (Jones et al. 1974) and 8α-hydroxy-Δ^9-THC (BenZvi et al. 1974) (Table 1, compound 1,2). Later on, transformation of 8α-; 8β- and 11-hydroxy-Δ^9-THC was studied (Burstein and Shoupe 1981), metabolism of 8α-hydroxy-Δ^9-THC was parallel to that of Δ^9-THC but the presence of 8β-hydroxy group was found to suppress the hydroxylation and oxidation at C-11 and to increase the β-oxidation of the side-chain (Harvey et al. 1980b).

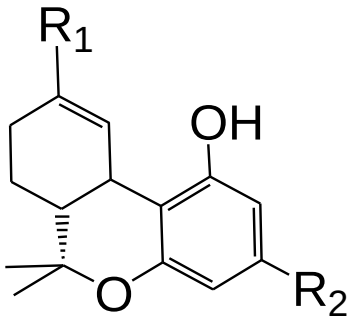
The *in vivo* metabolism of Δ^8-THC produced three alcohols, five diols, six triols, five monohydroxy acids, six dihydroxy acids, two substituted ketones, an epoxide, three dihydroxy metabolites and a glucuronide conjugate (Harvey and Paton 1980). Δ^{11}-THC was transformed into 26 metabolites produced by epoxidation among which 9α,11- and 9β,11-dihydroxyhexahydrocannabinols were the key metabolites (Harvey et al. 1980c). 11-oxo-Δ^8-tetrahydrocannabinol (11-oxo- Δ^8-THC) was identified from the mouse brain as a new *in vivo* metabolite of Δ^8-THC (Watanabe et al. 1980).

Metabolism of THC in monkey, guinea pig and dog

Purified Cytochrome P450 isozyme of monkey metabolized Δ^9-THC to 11-; 8α-; 8β- and 3'- hydroxy-Δ^9-THC (Table 1, compound 1, 2, 3, 6) (Matsunaga et al. 1995). Moreover, Δ^9-THC was also metabolized to some dihydroxy derivatives, such as 8α,11-; 8β,11-; 1',11-; 2',11-; 3',11- and 4',11-Δ^9-THC (Table 2, compound 9, 10, 11, 12, 13, 14) (Wall and Brine 1976).

Metabolism and autoradiography distribution showed the biotransformation of Δ^8-THC to the more potent and pharmacologically active 11-hydroxylated

Table 3. Mono-carboxylic acid metabolites of Δ^9-THC. (1) man, (2) rhesus monkey, (3) mouse, (4) rat, (5) rabbit, (6) guinea pig, (7) dog, (8) microorganisms.



No	R^1	R^2	Species								References
			1	2	3	4	5	6	7	8	
20	COOH	C_5H_{11}	+	+	+	+	+	+	+	-	(BenZvi and Burstein 1974, Halldin et al. 1982c, Harvey and Paton 1976, Harvey et al. 1978b, Harvey et al. 1980a, Kanter and Hollister 1978, Nordqvist et al. 1979a)
21	CH_3	COOH	-	-	-	-	-	+	-	-	(Harvey et al. 1980a)
22	CH_3	CH_2COOH	-	-	-	-	-	+	-	-	(Harvey et al. 1978b, Harvey et al. 1980a)
23	CH_3	C_2H_2COOH	+	+	+	-	-	+	-	+	(Halldin et al. 1982b, Harvey et al. 1978b, Harvey et al. 1980a, Nordqvist et al. 1979a, Robertson et al. 1978a)
24	CH_3	C_3H_6COOH	-	-	-	-	-	+	-	-	(Harvey et al. 1978b, Harvey et al. 1980a)
25	CH_3	C_4H_8COOH	-	-	-	-	-	-	-	+	(Robertson et al. 1978a)

metabolite in the monkey *Callithrix jacchus* (Just et al. 1975). The rhesus monkey metabolized Δ^8-THC to various monohydroxy and dihydroxy metabolites. 11-hydroxy-Δ^8-THC was the most abundant metabolite. Furthermore, all the side-chain hydroxy metabolites except 5'-hydroxy- Δ^8-THC were identified. The total 4'-hydroxy- derivative was present in about one-third and 3'-hydroxy- in about one-sixth of the amount of 11-hydroxy-Δ^8-THC. Only minor amounts of 1'- and 2'-hydroxy- Δ^8-THC were isolated (Halldin et al. 1979, Widman et al. 1979).

Several mono-, di- and trihydroxy derivatives were formed by *in vivo* liver metabolism of Δ^9-THC by guinea pigs. The 11-; 8α-; 8β-; 1'-; 2'- and 3'- were the monohydroxy derivatives (Table 1, compound 1,2 ,3 4, 5, 6), 8α,11-; 8β,11-; 2,11-; and 3,11-dihydroxy derivatives (Table 2, compound 9, 10, 12, 13), 2,8α,11- and 3,8α,11- were major trihydroxy derivatives (Table 2, compound 18) (Harvey et al. 1978b). Guinea pigs also produced large amounts of the monocarboyxlic acids (Table 3, compound 20, 21, 22, 23, 24), substituted side-chain carboxylic acid (Table 4, compound 32), mono- and dihydroxy-derivatives of Δ^9-THC-11-oic acid (Table 5, compound 41, 42, 43, 44). The major metabolic pathways involved were allylic and aliphatic hydroxylations, oxidation of alcohols to ketones and acids, β oxidative degradation of the pentyl side chain and conjugation with glucuronic acid (Harvey et al. 1978a, Harvey et al. 1980a).

Twenty-nine metabolites were reported in a study of Δ^8-THC metabolism by guinea pigs. The 1'-hydroxy- and 4'-oxo-Δ^8-THC-11-oic acid were new metabolites with other metabolites having β-oxidation at the side chain (Harvey and Paton 1981). Hepatic microsomes of guinea pig oxidized Δ^8-THC to 7α-OH- and 7β-OH-Δ^8-THC which were further converted into 7-oxo-Δ^8-THC but with different mechanisms in both cases (Narimatsu et al. 1988).

Isolated perfused dog lung metabolized Δ^9-THC into 3'-hydroxy- and 4'-hydroxy- derivatives (Table 1, compound 6, 7) as the major metabolites, whereas small amounts of 8α- and 8β-hydroxy-Δ^9-THC (Table 1, compound 2, 3) were also produced which are predominant metabolites identified from *in vitro* dog liver microsomal metabolism of Δ^9-THC (Widman et al. 1975a).

Table 4. Substituted side-chain carboxylic acid metabolites of Δ^9-THC (1) man, (2) rhesus monkey, (3) mouse, (4) rat, (5) rabbit, (6) guinea pig, (7) dog, (8) microorganisms.

			Species								References
No	Side-chain	Substituents	1	2	3	4	5	6	7	8	
26	COOH	11-OH, 8α-OH, 8β-OH, 8β, 11-di-OH	–	–	–	–	+	–	–	–	(Nordqvist et al. 1979a)
27	COOH	9-COOH	+	–	–	–	+	–	–	–	(Halldin et al. 1982a, Nordqvist et al. 1979a)
28	CH_2COOH	11-OH	+	–	–	–	–	–	–	–	(Halldin et al. 1982b)
29	CH_2COOH	8β-OH	+	–	–	–	+	–	–	–	(Halldin et al. 1982b, Nordqvist et al. 1979a, Nordqvist et al. 1979b)
30	CH_2COOH	9-COOH	+	–	–	–	–	–	–	–	(Halldin et al. 1982a)
31	C_2H_4COOH	11-OH	+	–	–	–	+	–	–	–	(Halldin et al. 1982b, Nordqvist et al. 1979b)
32	C_2H_4COOH	8β-OH	+	–	–	–	+	+	–	–	(Halldin et al. 1982b, Harvey et al. 1980a, Nordqvist et al. 1979a, Nordqvist et al. 1979b)
33	C_2H_4COOH	8β, 11-di- OH	–	–	–	–	+	–	–	–	(Nordqvist et al. 1979a)
34	C_2H_4COOH	9-COOH	+	–	–	–	+	–	–	–	(Halldin et al. 1982a, Nordqvist et al. 1979a, Nordqvist et al. 1974)
35	C_2H_4COOH	11-COOH	+	–	–	–	+	–	–	–	(Halldin et al. 1982a, Nordqvist et al. 1974)
36	C_3H_6COOH	8β-OH	+	–	–	–	–	–	–	–	(Halldin et al. 1982b)
37	C_3H_6COOH	9-COOH	+	–	–	–	–	–	–	–	(Halldin et al. 1982a)
38	C_4H_8COOH	9-COOH	–	–	–	–	+	–	–	–	(Nordqvist et al. 1979a, Nordqvist et al. 1979b)
39	CH=CH-COOH	9-COOH	+	–	–	–	+	–	–	–	(Halldin et al. 1982a, Nordqvist M. et al. 1979a)
40	CH_2-CH=CH-COOH	9-COOH	+	–	–	–	–	–	–	–	(Halldin et al. 1982b)

Metabolism of THC in mice and rat

Several derivatives have been synthesized by administering Δ^8- and Δ^9-THC to

Table 5. Hydroxy and dihydroxy derivatives of Δ^9-tetrahydrocannabinol-11-oic acid. (1) man, (2) rhesus monkey, (3) mouse, (4) rat, (5) rabbit, (6) guinea pig, (7) dog, (8) microorganisms

		Species								References
No	Position of OH	1	2	3	4	5	6	7	8	
41	8α-OH	–	–	+	+	–	+	–	–	(Harvey and Paton 1976, Harvey et al. 1978b, Harvey et al. 1980a)
42	1'-OH	+	–	–	–	+	+	–	–	(Burstein et al. 1972, Halldin et al. 1982b, Harvey et al. 1980a)
43	2'-OH	–	–	+	+	+	+	–	–	(Burstein et al. 1972, Halldin et al. 1982b, Harvey and Paton 1976, Harvey et al. 1978b, Harvey et al. 1980a, Nordqvist et al. 1979b)
44	3', 4'-di-OH	–	–	+	+	+	+	–	–	(Halldin et al. 1982b, Harvey and Paton 1976, Harvey et al. 1978b, Harvey et al. 1980a, Nordqvist et al. 1979b)
45	2',8α-, 3',8α-, 4',8α-di-OH	–	–	+	+	–	–	–	–	(Harvey and Paton 1976, Harvey and Paton 1978, Harvey et al. 1978b)

Table 6. Mono- and di-hydroxy derivatives of 8-oxo-Δ^9-tetrahydrocannabinol. (1) man, (2)rhesus monkey, (3) mouse, (4) rat, (5) rabbit, (6) guinea pig, (7) dog, (8) microorganisms.

		Species								References
No	Position of OH	1	2	3	4	5	6	7	8	
46	7-, 2'-, 3'-OH	–	+	–	–	–	+	–	–	(Harvey et al. 1977, Harvey et al. 1978b, Harvey et al. 1980a)
47	4'-OH	–	–	–	–	–	+	–	+	(Binder 1976, Harvey et al. 1980a)
48	2', 11-, 3', 11-, 4', 11-di-OH.	–	–	+	–	–	–	–	–	(Harvey and Paton 1978, Harvey et al. 1977, Harvey et al. 1978b)

male mice (Charles River CD-1) to predict metabolic pathways and to find new metabolites. Brown and Harvey (1988a) reported the metabolism of Δ^9-tetrahydrocannabiorcol (Δ^9-THCO) (Fig. 3d) and Δ^8-Tetrahydrocannabiorcol (Δ^8-THCO) into Δ^9-THC-11- and Δ^8-THCO-11-oic acid respectively. Sixteen metabolites were identified from n-hexyl-Δ^9-THC (Fig. 3d) and eleven from n-hexyl-Δ^8-THC (Brown and Harvey 1988b). Seven metabolites were formed from each Δ^9-tetrahydrocannabivarin (Δ^9-THCV) (Fig. 3d) and Δ^8-

tetrahydrocannabivarin (Δ^8-THCV). The major biotransformation pathway led to the production of 11-hydroxy-tetrahydrocannabivarin and their further oxidation to carboxylic acid metabolites, other metabolites were mainly the hydroxy derivatives of these compounds (Brown and Harvey 1988c). Metabolism of 2'-, 3'- and 4'-hydroxy-Δ^9-THC were also studied in mice. Hydroxylation at the allylic 11-position followed by oxidation to the carboxylic acid was found to be an important metabolic pathway (Harvey 1990). Little oxidative degradation of the pentyl side chain was found for 2'-hydroxy-Δ^9-THC (Table 1, compound 5) but abundant metabolism occurred by oxidative cleavage of the pentyl side chain from 3' and 4'-hydroxy-Δ^9-THC (Table 1, compound 6, 7) (Harvey 1989).

Harvey and Paton (1979) described the conversion of deuterium-labeled Δ^8-, Δ^{11}- and Δ^9-THC into a number of metabolites containing 1-3 additional groups at positions 2'; 3'; 4'; 8α; 8β and 11 with the only difference that Δ^{11}-THC did not undergo allylic hydroxylation at position 11. *In vivo* liver metabolites of Δ^9-THC were extracted from rats. The 11-; 8α-; 8β- and 3'- (Table 1, compound 1, 2, 3 and 6) were the monohydroxy-, 8α,11-; 8β,11- and 3',11- (Table 2, compound 9, 10, 13) were the major dihydroxy- derivatives (Harvey et al. 1978b). Rat liver microsomes transformed Δ^9-THC into 11-oxo- (Fig. 3e) and further reduction of this derivative produced 11-hydroxy-Δ^9-THC (Table 1, compound 1) (BenZvi and Burstein 1974, Burstein and Kupfer 1971a). Rat lever homogenates led to the formation of 11-hydroxy- (Table 1, compound 1) (Burstein and Kupfer 1971b) and 8α,11-dihydroxy-Δ^9-THC (Table 2, compound 9) (Burstein and Kupfer 1971a, Wall 1971, Wall et al. 1970).

Rat liver incubation of Δ^8-THC produced 11-hydroxy-; 7α,11- and 7β,11-dihydroxy-Δ^8-THC (Wall 1971). Purified cytochrome P450 isozymes from rat hepatic microsomes are reported to be able to catalyze the oxidation of 11-oxo-

Δ^8-THC to Δ^8-THC-11-oic acid (Watanabe et al. 1991). Sex related differences were observed in the oxidative metabolism of Δ^9-THC between male and female rats. Liver microsomes of male rats biotransformed Δ^9-THC into various metabolites unlike female rats in which it was mainly oxidized selectively to 11-hydroxy-Δ^9-THC (Table 1, compound 1) (Burstein and Kupfer 1971a, Narimatsu et al. 1991).

Biotransformation of THC in mammalian cells shows that cytochrome P450 enzymes play an important role in the hydroxylation of THC. THC undergoes allylic hydroxylation at C-11 to form 11-hydroxy-THC which is the most common metabolite in almost every species studied. Allylic hydroxylation is also common at position 8 to give 8α- and 8β-hydroxy-THC. Other than allylic hydroxylations there are a number of monohydroxy and dihydroxy metabolites formed, being hydroxylated at all the positions of the alkane side chain of THC. There is however considerable species variation in the position of substitution. In addition to monohydroxy and dihydroxy, some polyhydroxylated metabolites have also been found in mice with hydroxyl substitution at position 2' and 3'. In monkeys and dogs, hydroxyl substitution was more common on the positions 3' and 4' while the monkey also formed minor amounts of metabolites having hydroxylation on position 1' and 2'. The major metabolic pathway of THC (Fig. 4) after monohydroxy metabolism is further oxidation to either a carbonyl compound or, in the case of the primary alcohols to a carboxylic acid, followed by glucuronidation of the acid. Following this biotransformation pathway there are a number of THC acids which are formed in man, rats, rabbits and guinea pigs where the acids link with glucuronide and become the major urinary excretion product.

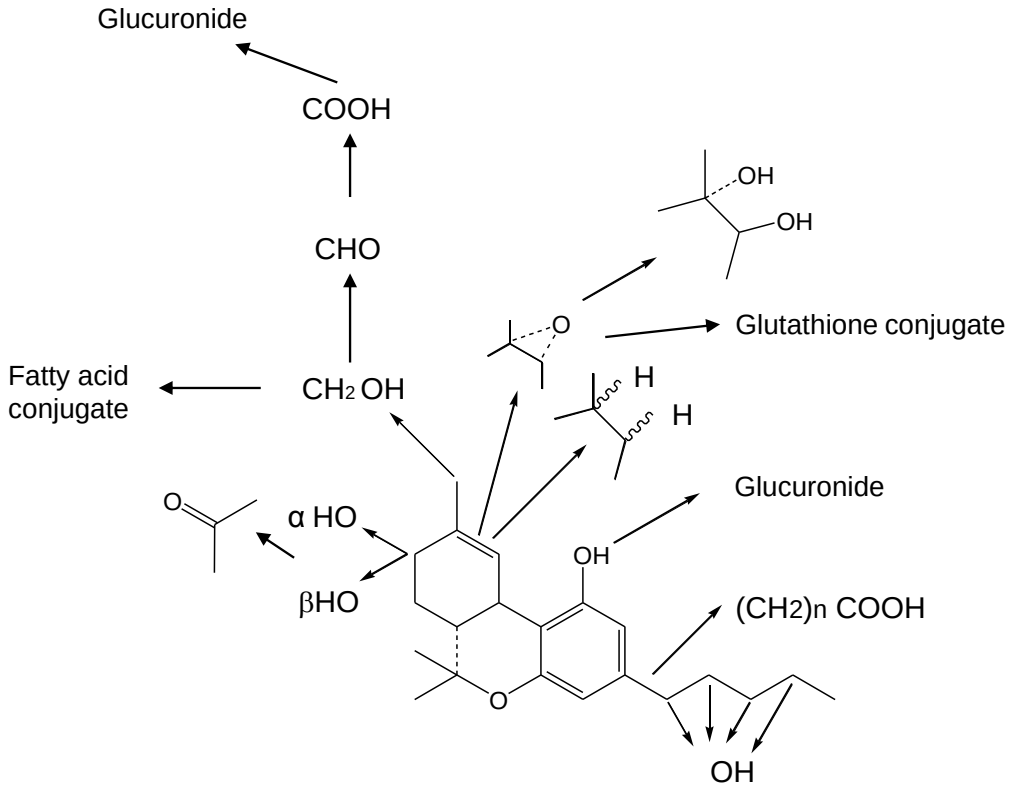


Figure 4: Metabolic pathway of Δ^9-THC

Biotransformation of Cannabidiol

Biotransformation of cannabidiol (CBD) has extensively been studied in mice and rats. Fourteen metabolites were isolated from the liver of mice after the administration of CBD. The most significant biotransformation step was glucoronide conjugation and to a lesser extent CBD-11-oic-acid was formed (Martin et al. 1977). Eight monohydroxy (Martin et al. 1976b) and ten dihydroxy metabolites were found in the *in vitro* transformation of CBD in rats

(Martin et al. 1976a), in both cases mainly 11-hydroxylation was formed with further hydroxylation at C-4 of the side chain. Over fifty metabolites of CBD were identified with considerable species variation in dog, rat and human urine. Thirty three metabolites were identified from the urine of dystonic patients treated with CBD. The major metabolic pathway was the hydroxylation and oxidation at C-11 and further hydroxylation in the pentyl and propyl groups produced 1'-; 2'-; 3'; 4'- and 10-hydroxy derivatives of CBD-11-oic acid. Acids were formed by β-oxidation and further biotransformations from the pentyl side-chain resulted in the formation of the oxidized metabolite CBD-11-oic acid (Harvey and Mechoulam 1990). In dog unusual glucoside conjugates were found of the metabolites 4'-; 5'-hydroxy- and 8-oxo-CBD. Other metabolites in all three species were mainly acids. Side-chain hydroxylated derivatives of CBD-11-oic acid were abundant in human urine (Harvey et al. 1991). CBD followed almost the same pattern of biotransformation as observed in Tetrahydrocannabinol, with several variations in pathways caused by different species. The monohydroxylation of the side chain and also at position 11 and 8 found in CBD is quite similar to THC. Dihydroxy compounds are also reported but unlike THC no trihydroxy metabolites are found in any species treated with CBD. A large number of metabolites are formed by β-oxidation of the side chain and Glucoronidation of CBD.

Biotransformation of Cannabinol

Cannabinol was metabolized by rat liver into side chain hydroxylated compounds: the 2'-; 3'-; 4'- and 5'-hydroxy were isolated as minor metabolites and 11-hydroxy-CBN was formed as the major metabolite (Widman et al. 1975b). Cannabinol-11-oic acid was found as a major compound from rat feces (Yisak et al. 1977). The administration of ^{14}C-labeled cannabinol to rats resulted in mono- and dihydroxy metabolites. The monohydroxy 11- and 4'-

hydroxy- (Lindgren et al. 1977, Yisak et al. 1977) and the dihydroxy- 1',11-; 4',11-; 2',11- and 3',11-dihydroxycannabinol were the most abundant compounds (Fonseka and Widman 1977, Yisak et al. 1977).

In rabbits, 4'-hydroxy-CBN was found as a major compound together with smaller amounts of 5'- and 3'-hydroxy-CBN (Widman et al. 1975b). In mice, the 11-position and the side chain of cannabinol was hydroxylated followed by further oxidation to acidic metabolites (Burstein and Varanelli 1975). Biotransformation of CBN also follows the same pattern like other cannabinoids but unlike other cannabinoids allylic hydroxylation of the terpene ring is absent in the metabolism of CBN. The 11-hydroxylation is the major metabolic route and further oxidation of this monohydroxy compound produces a common acidic metabolite, CBN-11-oic acid.

Biotransformation of cannabinoids by fungi and bacteria

Robertson et al. (1978a, 1978b) screened more than 100 species of fungi and bacteria to investigate microbial transformations of the four common cannabinoids, namely CBD, CBN, Δ^8-THC and Δ^9-THC. *Syncephalastrum racemosum* and *Mycobacterium rhodochrous* partially degraded the n-pentyl side chain of all these four compounds. Carboxylic acid and alcohol side chain derivatives were found to be the major metabolites. Among side chain derivatives, the 4'-hydroxy- metabolites were the most abundant compounds in the transformation of CBD, CBN, Δ^8-THC and Δ^9-THC by *Syncephalastrum racemosum* (Robertson and Lyle 1975).

Δ^8-Tetrahydrocannabinol was hydroxylated at the ring system and at the side chain by fermentation with *Pellicularia filamentosa, Streptomyces lavendulae* or *P. filamentosa* and yielded the compounds 7β,3'- and 7β,4'-dihydroxy-Δ^8-THC. *Streptomyces lavendulae* leads to the formation of 7α–hydroxy-, 7α,2'-;

7α,3'-; 7α,4'-dihydroxy- Δ^8-THC and 4'-hydroxy-7-oxo-Δ8-THC (Vidic et al. 1976).

Binder and Meisenberg (1978) indentified 51 fungal and bacterial strains which actively transformed Δ^9-THC into 11-; 8α-; 8β-; 3'- and 4'-hydroxy- (Table 1, compound 1, 2, 3, 6) and 4',11-dihydroxy-Δ^9-THC (Table 2, compound 14). *Cunninghamella blakesleeana* also produced 4'-hydroxy-8-oxo-Δ^9-THC (Table 6, compound 47) (Binder 1976).

Binder and Popp (1980) further studied the metabolic transformations of Δ^9-THC by using cultures of *Fusarium nivale, Gibberella fujikuroi* (Ascomycetes) and *Thamnidium elegans* (Phycomycetes). A number of metabolites were isolated from these species after which they were partly purified and their structures elucidated by combined gas chromatography/mass spectrometry. *Thamnidium elegans* formed 11-; 8α-; 8β-; and 1'-hydroxy- (Table 1, compound 1, 2, 3, 4) and 2',8β-dihydroxy-Δ^9-THC (Table 2, compound 16). *Fusarium nivale* and *Gibberella fujikuroi* both converted Δ^9-THC to the metabolites 2'-, 3'- and 4'-hydroxy-Δ^9-THC (Table 1, compound 5, 6, 7). These results show that there are two different enzyme systems capable of hydroxylating the substrate. System 1, which is common to the "*Fusarium*" type and microorganisms are restricted in its hydroxylating capacity to the side chain C-atoms 2', 3' and 4' of cannabinoids. In addition to this 'aliphatic hydroxylase' *Thamnidium elegans* posseses an 'allylic hydroxylase' capable of hydroxylating Δ^9-THC in positions 1', 8 and 11.

Microorganisms show similarities with mammalian hydroxylation of cannabinoids. Cannabinoids undergo allylic hydroxylation at C-11 to form 11-hydroxy- derivatives. Allylic hydroxylation is also seen at position 8 to give 8α- and 8β-hydroxy derivatives but unlike mammals, hydroxylation has also been found at position 7 (only found in guinea pig in mammals) and further oxidation

to form a carbonyl compound. In microorganisms, side chain degradation is found as one of the major metabolic pathways of cannabinoids. Side chain hydroxyl and carboxyl substitution is also common; hydroxylation is more likely on C-atom 2', 3' and 4' with their corresponding dihydroxy metabolites. Trihydroxy derivatives of naturally occurring cannabinoids are not found in bacteria or fungi. Side chain acid derivatives are present but carboxylation of the monoterpene moiety and glucoronidation is not reported.

Plant biotransformation of cannabinoids

Cannabielsoin (CBE) and its diastereoisomers have been isolated from the suspension cultures of *C. sativa*, *Saccharum officinarum* and tissue culture of *C. sativa*, after the administration of cannabidiol (Braemer and Paris 1987, Tanaka et al. 1997). Δ^9-THC was converted into cannabicoumaronon by a cell suspension culture of *C. sativa* (Braemer and Paris 1987). Tissue culture of *Pinelli ternatata* transformed Δ^8-THC and CBN into their glucopyranoside derivatives Δ^8-THC-2'-O-β-D-Glucopyranoside (Tanaka et al. 1997) and CBN-2'-O-β-D-Glucopyranoside, respectively (Tanaka et al. 1993).

Discussion and conclusions

Despite having useful psychomimetic and pharmacological activities, research into cannabis has been mainly focused on its use as a recreational drug and the subsequent legal aspects controlling the possession of this material. Some of this research has been conducted on the metabolism of cannabinoids in mammalian systems, leading to a better understanding of their pharmacodynamics and pharmacokinetics. A large library of THC isomers and their derivatives have been developed by using mammalian cytochrome P450 systems, which is a successful approach to finding metabolic pathways but little is known about the activity and interaction of these metabolites with the CB1 and CB2 receptors..

Perhaps the industrial scale production of potential compounds by means of mammalian cells or tissue is an expensive tool and thus an obstacle to develop cannabinoid based drugs. There are a number of other sources which can be used to enlarge the library of active metabolites obtained from the transformation of cannabinoids. Cannabinoids have been successfully transformed by fungal and bacterial strains, while there are only a few reports on the biotransformation with plant cell cultures. Microorganisms mimic some of the mammalian biotransformation pathways and their enzyme systems have great potential to produce improved or novel compounds in large quantities keeping production at a low cost.

Bacterial cytochrome P450 (monooxygenases) enzymes have the ability to hydroxylate exogenous and endogenous compounds at different positions. In the case of cannabinoid transformations, hydroxylation is likely to produce more polar cannabinoids which might be more suitable for medicinal use than parent compounds, which generally tend to accumulate in fat tissues. The significant role of the alkane side chain has been reported in the pharmacological action of THC (Billy et al. 1995) and further oxidation may change the chemistry of the side chain and lead to enhanced or modified activity of the compound. By taking into account these findings, we used alkane degrading bacterial strains for the transformation of Δ^9-THC and found eight major metabolites produced in mg scale. All these transformations were limited to the side chain and included two carboxylic acid derivatives formed by oxidation and β-oxidation of the terminal hydroxyl group of 5$^{'}$-hydroxy-Δ^9-THC (Rashidi et al. 2009). Numbers of new lead candidates are possible to obtain by using the enzymatic systems of bacterial, yeast and plant cells in cannabinoid transformation in combination with advanced chromatographic and analytical techniques.

There are more than 72 known cannabinoids but few reports on the metabolism or transformation of any other natural cannabinoid, except Δ^9-THC, CBN and CBD. Low availability of most of the cannabinoids limits the study of their pharmacological properties. There is a demand for non-psychoactive cannabinoids and their metabolites produced after biotransformation, as this might be a source of new compounds with interesting pharmacological profiles. Further studies with different microorganisms and a diverse range of cannabinoids is thus of great interest. New cannabinoid derivatives devoid of the psycho activity with only the desired profile of pharmacological activity are of great interest to the scientific community.

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