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In Vivo Metabolism of 11-Hydroxy-Δ^8-tetrahydrocannabinol to Two Carboxylic Acids and the Effects of SKF 525-A, Cobaltous Chloride and Phenobarbital on Their Formation in Rats

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Two carboxylic acids were isolated as the major metabolites from the liver of rats injected with 11-hydroxy-Δ^8-tetrahydrocannabinol (11-OH-Δ^8-THC). One of the metabolites was identified as Δ^8-THC-11-oic acid, and the other was assumed to be 7-hydroxy-Δ^8-THC-11-oic acid. The effects of SKF 525-A, cobaltous chloride and phenobarbital on the contents of these metabolites in the rat liver were studied. Pretreatment with SKF 525-A and cobaltous chloride resulted in significant decreases of the contents of these metabolites (by 73 and 70% for Δ^8-THC-11-oic acid, and by 91 and 89% for 7-hydroxy-Δ^8-THC-11-oic acid, respectively). On the other hand, the level of 7-hydroxy-Δ^8-THC-11-oic acid was increased, but that of Δ^8-THC-11-oic acid remained unchanged by pretreatment with phenobarbital. These results suggest that the metabolism of 11-OH-Δ^8-THC to Δ^8-THC-11-oic acid and 7-hydroxy-Δ^8-THC-11-oic acid is mediated in part by the microsomal monooxygenase system *in vivo*.

Keywords——Δ^8-tetrahydrocannabinol; 11-hydroxy-Δ^8-tetrahydrocannabinol; Δ^8-tetrahydrocannabinol-11-oic acid; 7-hydroxy-Δ^8-tetrahydrocannabinol-11-oic acid; SKF 525-A; cobaltous chloride; phenobarbital; metabolic inhibition; microsomal monooxygenase

Δ^8-Tetrahydrocannabinol (Δ^8-THC), which is an active component of marihuana,[2] is known to be transformed to 11-hydroxy-Δ^8-tetrahydrocannabinol (11-OH-Δ^8-THC) in the rat *in vitro*[3] and *in vivo*.[4] Further oxidation of 11-OH-Δ^8-THC at the 11-position results in the formation of the 11-carboxylic acid in experimental animals.[5] This metabolic pathway involves the oxidation of alcohol to aldehyde and further to carboxylic acid.[6] Recently, we reported that 11-OH-Δ^8-THC was metabolized to 11-oxo-Δ^8-THC by a liver microsomal enzyme system involving cytochrome P-450.[7] Such enzymatic reaction also occurs in rats and mice.[8] These results stimulated interest in the contribution of the monooxygenase system to the biotransformation of 11-OH-Δ^8-THC to Δ^8-THC-11-oic acid through 11-oxo-Δ^8-THC *in vivo*.

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When radio-labeled Δ^8-THC or Δ^9-THC is injected into rats, it is well known that high radioactivity appears in the liver as well as in the lung and kidney.[9] In addition, Δ^9-THC is known to be metabolized mainly in the liver, rather than in other tissues.[10] Estevez *et al.* also found an acidic metabolite of 11-OH-Δ^8-THC in rat liver.[11] Such evidence suggests that it would be advantageous to use the liver for following up acidic metabolites.

In the present study, we intended to investigate the participation of the microsomal monooxygenase system in the *in vivo* metabolism of 11-OH-Δ^8-THC to Δ^8-THC-11-oic acid in rat liver.

Materials and Methods

Chemicals——11-OH-Δ^8-THC and Δ^8-THC-11-oic acid were prepared by the method reported previously.[12] SKF 525-A was kindly supplied by Smith Kline and French Lab. All other chemicals used were obtained commercially.

Drug Administrations——Male Wistar rats (200—250 g) were injected intravenously with 5 mg/kg of 11-OH-Δ^8-THC dissolved in propylene glycol–ethanol (1: 1). In pretreatment studies, 11-OH-Δ^8-THC was injected 20—24 hr after the last injection of sodium phenobarbital (75 mg/kg, *i.p.*, once a day for 4 days) or cobaltous chloride (30 mg/kg, *s.c.*, twice a day for 3 days). SKF 525-A (25 mg/kg, *i.p.*) was administered 30 min before the injection of 11-OH-Δ^8-THC.

Isolation and Determination of the Metabolites——Rats were killed by a blow on the head at 15, 30, 60 or 120 min after the injection of 11-OH-Δ^8-THC. The liver was then removed and homogenized with an equal volume of water. It was extracted three times with five volumes of methanol. After removal of the solvent by evaporation, the extract was dissolved in 50 ml of ethylether and shaken three times with 20 ml of 0.1 N NaOH. The combined alkaline extract was acidified with 1 N HCl and extracted three times with 50 ml of ethylether. Each metabolite was finally purified by preparative thin-layer chromatography with a solvent system of benzene–ethanol–diethylamine (20: 10: 1). The zones corresponding to the metabolites detected by spraying Fast Blue BB salt were scraped off and extracted twice with 5 ml of ethanol. The metabolites were determined by the method previously reported, using Fast Blue BB salt.[13] The methyl esters of these metabolites were characterized by mass spectrometry. The mass spectra were obtained using a JMS-01 SG spectrometer. The ionizing energy was 20 eV.

Results and Discussion

Identification of Two Carboxylic Acid Metabolites

Two carboxylic acid metabolites were found as the major metabolites of 11-OH-Δ^8-THC in all the rat livers (taken at 15 to 120 min after the injection of 11-OH-Δ^8-THC) by thin-layer chromatography (*Rf* values were 0.35 and 0.25). The mobility of a less polar metabolite (*Rf*=0.35) in thin-layer chromatography was the same as that of synthetic Δ^8-THC-11-oic acid. Mass spectra of the methyl esters of the metabolites with higher and lower *Rf* values showed the parent ions at *m/e* 358 and 374, respectively. These results indicate that one of the metabolites (*Rf*=0.35) was Δ^8-THC-11-oic acid and the other (*Rf*=0.25) should be its hydroxylated derivative. Both metabolites showed a predominant ion at *m/e* 231, suggesting that a hydroxy group is introduced in the cyclohexene ring of Δ^8-THC on the basis of known mass fragmentations of Δ^8-THC and Δ^9-THC.[14] The presence of another fragment ion at *m/e* 297 (M^+-77) showed the elimination of the 11-$COOCH_3$ and H_2O.

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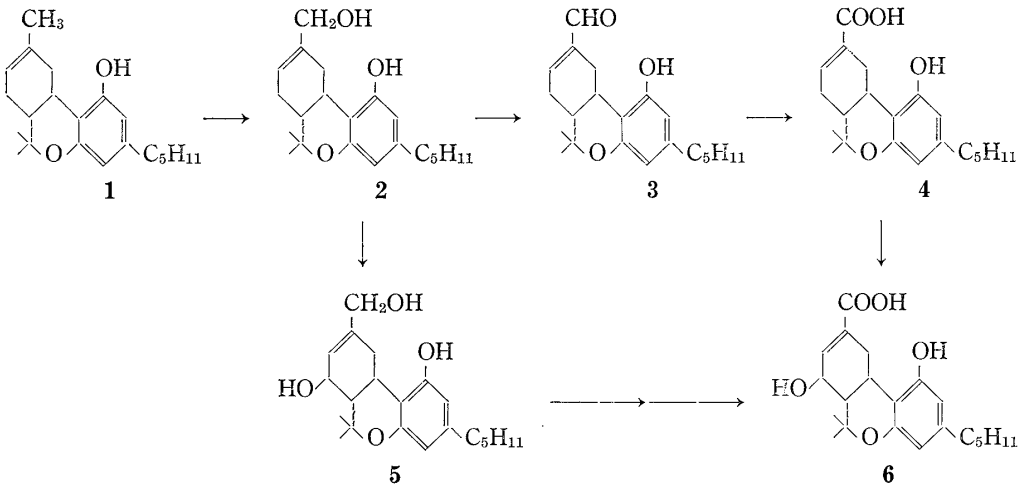

1: Δ^8-THC, 2: 11-OH-Δ^8-THC, 3: 11-oxo-Δ^8-THC, 4: Δ^8-THC-11-oic acid
5: 7,11-diOH-Δ^8-THC, 6: 7-OH-Δ^8-THC-11-oic acid

Chart 1. Possible Pathways for the Formation of 7-Hydroxy-Δ^8-tetrahydrocannabinol-11-oic Acid

A similar fragmentation has been reported by Wall with 7,11-dihydroxy-Δ^8-THC (loss of CH_2-OH and H_2O).[3] The structure of this metabolite was thus assigned as 7-hydroxy-Δ^8-THC-11-oic acid, which is a novel metabolite of Δ^8-THC. The possible pathways for the formation of 7-hydroxy-Δ^8-THC-11-oic acid from 11-OH-Δ^8-THC are shown in Chart 1 *via* (2)⟶(3)⟶(4)⟶(6) and (2)⟶(5)⟶(6).

Time Course of the Contents of the Two Carboxylic Acid Metabolites in Rat Liver

The time course of the contents of the two carboxylic acid metabolites in rat liver is shown in Fig. 1. The content of Δ^8-THC-11-oic acid increased up to 30 min, then decreased slightly from 30 min to 120 min after the injection of 11-OH-Δ^8-THC. On the other hand, the content of 7-hydroxy-Δ^8-THC-11-oic acid increased gradually up to 120 min. The level of total carboxylic acid thus remained almost constant from 30 to 120 min. The results suggest that 7-hydroxy-Δ^8-THC-11-oic acid is probably derived from Δ^8-THC-11-oic acid in the rat.

Pretreatment Studies with SKF 525-A, Cobaltous Chloride and Phenobarbital

In the previous paper,[7] we reported that rabbit liver microsomes catalyzed the oxidation of 11-OH-Δ^8-THC to 11-oxo-Δ^8-THC, and that SKF 525-A and cobaltous chloride caused

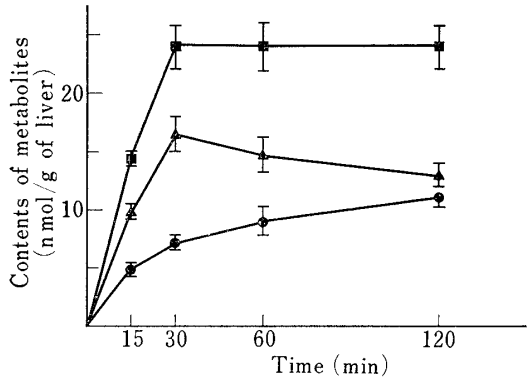

Fig. 1. Contents of Carboxylic Acid Metabolites of 11-Hydroxy-Δ^8-tetrahydrocannabinol in Rat Liver

—■—: total carboxylic acid metabolites,
—▲—: Δ^8-THC-11-oic acid,
—●—: 7-hydroxy-Δ^8-THC-11-oic acid.

Table I. Effects of SKF 525-A, Cobaltous Chloride and Phenobarbital on the Contents of Carboxylic Acid Metabolites of 11-Hydroxy-Δ^8-tetrahydrocannabinol in the Rat Liver[a]

	Δ^8-THC-11-oic acid	7-Hydroxy-Δ^8-THC-11-oic acid
Control	15.02±0.89	7.58±0.21
SKF 525-A	4.07±0.44[c]	0.69±0.08[c]
$CoCl_2$	4.56±0.57[c]	0.86±0.19[c]
Phenobarbital	14.56±0.99	11.22±0.71[b]

a) See "Materials and Methods" for the conditions. The liver was removed 30 min after the injection of 11-OH-Δ^8-THC. The results are means (nmol/g of liver)±S.E. from four rats.
b) $p<0.05$.
c) $p<0.01$.

a significant inhibition of this enzymatic reaction. Therefore, we examined the inhibitory effects of SKF 525-A and cobaltous chloride, and also the stimulatory effect of phenobarbital, on the *in vivo* metabolism of 11-OH-Δ^8-THC to the carboxylic acids described above. Pretreatment with SKF 525-A and cobaltous chloride, known inhibitors of microsomal monooxygenase,[15] resulted in significant decreases of the contents of both acidic metabolites (by 73 and 70% for Δ^8-THC-11-oic acid, and by 91 and 89% for 7-hydroxy-Δ^8-THC-11-oic acid, respectively) in the rat liver at 30 min after the injection of 11-OH-Δ^8-THC (Table I). The results are not conclusive but strongly suggest that SKF 525-A and cobaltous chloride inhibit not only hydroxylation at the 7-position but also aldehyde formation at the 11-position, which is involved in the metabolic route to the carboxylic acid metabolites. This supports the participation of microsomal monooxygenase in the *in vivo* metabolism of 11-OH-Δ^8-THC to 11-oxo-Δ^8-THC. However, this does not strictly exclude the possibility that the dehydrogenase system may also be responsible in part for the biotransformation of 11-OH-Δ^8-THC to 11-oxo-Δ^8-THC.

On the other hand, the level of 7-hydroxy-Δ^8-THC-11-oic acid was increased, but that of Δ^8-THC-11-oic acid remained unchanged by pretreatment with phenobarbital, which is a known inducer of microsomal monooxygenase (Table I).

In connection with the present study, it is well known that liver microsomes are capable of oxidizing primary alcohols such as ethanol,[16] and this microsomal ethanol oxidizing system (MEOS) also participates in ethanol oxidation *in vivo*.[17] Recently, the involvement of cytochrome P-450 in MEOS has been suggested by some investigators.[18] At present, however, it is not clear whether the microsomal enzyme system catalyzing the oxidation of 11-OH-Δ^8-THC to 11-oxo-Δ^8-THC is the same as the MEOS or not.

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