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Spectral Interaction of 11-Hydroxy-Δ^8-tetrahydrocannabinol with Rabbit and Rat Liver Microsomal Cytochrome P-450

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11-Hydroxy-Δ^8-tetrahydrocannabinol, which is an active metabolite of Δ^8-tetrahydrocannabinol, produced a spectral change on interacting with liver microsomes of rabbits and rats (λ_{max} 395 nm; λ_{min} 428 nm). The apparent dissociation constants (Ks) were found to be 402.1 and 76.6 μM for microsomes of untreated rabbits and rats, respectively. Pretreatment with phenobarbital enhanced the spectral change caused by 11-hydroxy-Δ^8-tetrahydrocannabinol with microsomes of both species, as well as increasing the cytochrome P-450 content. Furthermore, 11-hydroxy-Δ^8-tetrahydrocannabinol interfered with the spectral change caused by Δ^8-tetrahydrocannabinol or hexobarbital, but not with that caused by aniline. These results are consistent with the view that 11-hydroxy-Δ^8-tetrahydrocannabinol is further metabolized by the microsomal monooxygenase system involving cytochrome P-450.

Keywords——spectral interaction; tetrahydrocannabinol; hydroxytetrahydrocannabinol; metabolism of cannabinoids; P-450

It is well established that Δ^8- and Δ^9-tetrahydrocannabinol (Δ^8- and Δ^9-THC) are metabolized to the corresponding 11-hydroxy derivatives (11-OH-Δ^8- and Δ^9-THC) by the microsomal monooxygenase system.[2–4] Both cannabinoids elicited the characteristic type I spectral change with rat liver microsomes.[5–7] This spectral change is associated with the binding of these cannabinoids to cytochrome P-450, the terminal oxidase of the microsomal monooxygenase system. 11-OH-Δ^9-THC is known to be further metabolized to 8,11-dihydroxy-Δ^9-THC and carboxylic acid metabolites in animals,[8–10] and Esteves *et al.* have reported that levels of 7,11-dihydroxy-Δ^8-THC and carboxylic acid metabolites in rat liver after administration of 11-OH-Δ^8-THC are significantly reduced by pretreatment with SKF-525A, a known inhibitor of microsomal monooxygenase.[11] This evidence suggests that the metabolism of 11-OH-Δ^8-THC is catalyzed by microsomal monooxygenase. In addition, we recently found that microsomal oxygenase also catalyzes the formation of 11-oxo-Δ^8-THC from 11-OH-Δ^8-THC.[12] In contrast, Kupfer *et al.* suggested that 11-OH-Δ^8-THC did not interact with cytochrome P-450 of rat liver microsomes and that other enzymes might be involved in its further metabolism.[6] The present study was undertaken to re-examine the spectral interaction between rabbit and rat liver microsomal cytochrome P-450 and 11-OH-Δ^8-THC using higher concentrations than that used by Kupfer *et al.*

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Materials and Methods

Male albino rabbits (2.0—3.0 kg) and male Wistar rats (150—200 g) were used. For phenobarbital (PB) treatment, sodium PB (in 0.9% NaCl) was injected subcutaneously into the backs of animals at a dose of 20 mg/kg (rabbits) or intraperitoneally at a dose of 75 mg/kg (rats), twice a day for 4 days. Animals were fasted for and sacrificed at 20—24 hr after the last injections. Water was available at all times. Microsomes were prepared from 1.15% KCl liver homogenate (1: 4 w/v) by differential centrifugation as described previously,[12] and finally suspended in 0.1 M potassium phosphate buffer (pH 7.4). Δ^8-THC was prepared as described in the previous report,[13] and 11-OH-Δ^8-THC was synthesized by SeO_2 oxidation of Δ^8-THC.[14,15] The gas chromatographic purities of these cannabinoids were shown to be more than 98%. Difference spectra were measured with a Union SM-401 spectrophotometer. Δ^8-THC and 11-OH-Δ^8-THC in ethanol were added to a sample cuvette and an equal amount of ethanol was added to a reference cuvette. In interference experiments, 11-OH-Δ^8-THC was added to both the reference and sample cuvettes before addition of Δ^8-THC, hexobarbital or aniline to a sample cuvette. Cytochrome P-450 was determined by the method of Omura and Sato.[16] Protein concentration was measured by the method of Lowry *et al.*[17]

Results

When added to a suspension of liver microsomes from both untreated and PB-treated rabbits and rats, 11-OH-Δ^8-THC elicited a concentration-dependent type I spectral change (λ_{max} 395 nm; λ_{min} 428 nm), as exemplified by the interaction with rabbit liver microsomes shown in Fig. 1. Pretreatment of rabbits with PB enhanced the amplitude of the spectral change of microsomes with 11-OH-Δ^8-THC as well as increasing the amount of cytochrome P-450, as shown in Fig. 1B. A similar effect was also observed in rats on PB treatment. On the other hand, addition of low concentrations (<25 μM) of 11-OH-Δ^8-THC to untreated

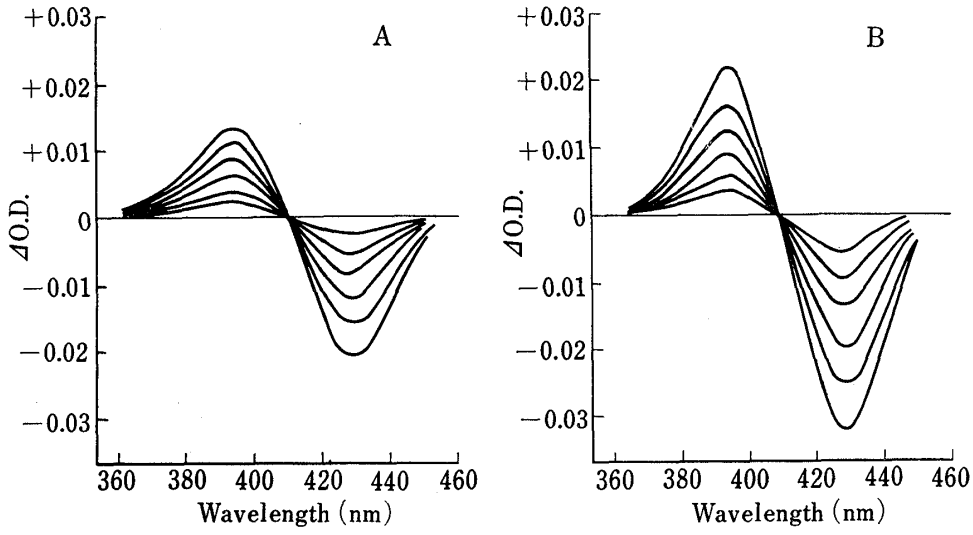

Fig. 1. Difference Spectra of 11-OH-Δ^8-THC with Rabbit Liver Microsomes

A: Untreated microsomes. B: PB-treated microsomes. Concentrations of 11-OH-Δ^8-THC were: A, (1) 48.1 μM; (2) 96.2 μM; (3) 192.4 μM; (4) 288.6 μM; (5) 384.8 μM; (6) 577.2 μM, and B, (1) 48.1 μM; (2) 96.2 μM; (3) 144.3 μM; (4) 240.5 μM; (5) 336.7 μM; (6) 577.2 μM, respectively. The protein concentration of both microsomal suspensions was 1.3 mg/ml.

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Table I. Binding Constants of Δ^8-THC and 11-OH-Δ^8-THC with Liver Microsomes of Rabbits and Rats[a]

	Δ^8-THC		11-OH-Δ^8-THC	
	K_s (μM)	A_{max}/P-450	K_s (μM)	A_{max}/P-450
Rabbit (3)[b] (untreated)	88.7± 4.5	0.051±0.07	402.1±36.2	0.048±0.002
Rabbit (3) (PB-treated)	148.5±12.3[c]	0.042±0.006	493.9±13.1	0.051±0.002
Rat (4) (untreated)	10.1± 0.1	0.026±0.001	76.6± 3.8	0.015±0.001
Rat (3) (PB-treated)	7.7± 0.7	0.030±0.001	82.9± 1.8	0.014±0.001

a) The concentration of microsomal protein used in this study was 1.3 mg/ml. The results are expressed as the means±S.E.
b) Numbers in parentheses represent the numbers of experiments.
c) Significantly different ($p<0.05$) from the untreated group.

microsomes of both species did not produce a significant spectral change, but clearly produced a type I spectral change with PB-treated microsomes. When 11-OH-Δ^8-THC was added at mM levels, however, it caused aggregation of the microsomes. The K_s values for 11-OH-Δ^8-THC were determined graphically to be 402.1±36.2 and 76.6±3.8 μM for microsomes from untreated rabbits and rats, and 493.9±13.1 and 82.8±1.8 μM for PB-treated microsomes, respectively (Table I). Table I also shows the maximum difference of spectral change (Δ O.D. 395—428 nm) per nmole of cytochrome P-450 for untreated and PB-treated rabbits and rats. In addition, 11-OH-Δ^8-THC interfered with the spectral change of Δ^8-THC with liver microsomes from PB-treated rabbits, as shown in Fig. 2. 11-OH-Δ^8-THC (121.2 μM) also inhibited (60%) the type I spectral change with hexobarbital (1 mM) using liver microsomes from PB-treated rats. These inhibitions were found in untreated microsomes as well as in PB-treated microsomes. However, 11-OH-Δ^8-THC (121.2 μM) did not affect the type II spectral change with aniline (1 mM) using the same microsomal suspension.

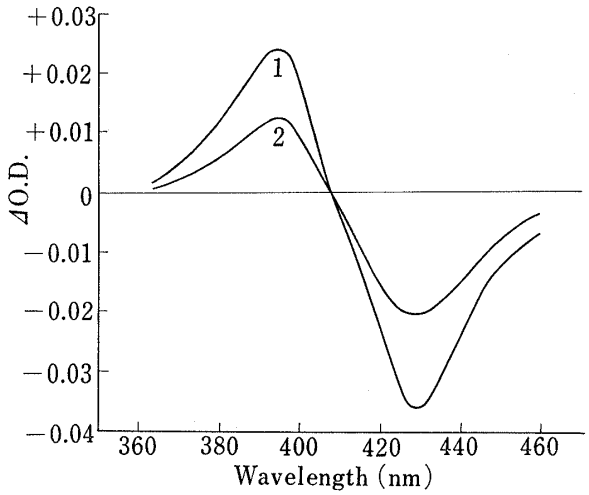

Fig. 2. The Effect of 11-OH-Δ^8-THC on the Spectral Change produced by Δ^8-THC with Rabbit Liver Microsomes

1: Δ^8-THC (212 μM) was added. 2: 11-OH-Δ^8-THC (240 μM) was added before addition of Δ^8-THC (212 μM). PB-treated microsomes were used (1.3 mg protein/ml).

Discussion

Recently, we reported that 11-OH-Δ^8-THC was metabolized to 11-oxo-Δ^8-THC by the microsomal oxygenase system involving cytochrome P-450 in rabbit liver.[12] On the other hand, Kupfer *et al.* reported that 11-OH-Δ^8-THC did not interact with cytochrome P-450 of rat liver microsomes, and suggested that other enzymes might be involved in its further metabolism.[6] In the present study, we clearly showed that 11-OH-Δ^8-THC elicited a type I spectral change with rabbit and rat liver microsomal P-450, and interfered with the spectral change caused by Δ^8-THC or hexobarbital, which are type I compounds, but not with that

caused by aniline, a type II compound. These observations suggest that 11-OH-Δ^8-THC and hexobarbital bind to the same cytochrome P-450. The major difference of our system from that of Kupfer *et al.* appears to be the use of higher concentrations of 11-OH-Δ^8-THC in the present study. Addition of lower concentrations (<25 μM) of 11-OH-Δ^8-THC to untreated rabbit and rat microsomes did not produce any significant spectral change, although it produced a type I spectral change with PB-treated microsomes containing higher levels of P-450.

It is well known that various substrates of the microsomal monooxygenase system bind to cytochrome P-450 and produce spectral changes.[18] The present results thus afford further support for the participation of rabbit and rat liver microsomal cytochrome P-450 in the further metabolism of 11-OH-Δ^8-THC. However, the present observations also show that the affinity of 11-OH-Δ^8-THC for cytochrome P-450 (K_s μM=402.1 for rabbits and 76.6 for rats) is considerably lower than that of Δ^8-THC (88.7 for rabbit and 10.1 for rats).

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Studies on Peptides. XCVI.[1,2] Behavior of S-Acetamidomethylcysteine Sulfoxide under Deprotecting Conditions in Peptide Synthesis

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The sulfoxide of Boc–Cys(S–acetamidomethyl)–OH was prepared by oxidation with sodium perborate. Mercuric acetate and iodine failed to cleave the S-protecting group from the sulfoxide. Hydrogen fluoride and methanesulfonic acid partially converted the sulfoxide to S-*p*-methoxyphenylcysteine in the presence of anisole. A reducing reagent, thiophenol, converted the sulfoxide to acetamidomethyl phenyl sulfide and N^{α}-Boc–S–(phenylthio)cysteine.

Keywords——S-acetamidomethylcysteine sulfoxide; mercuric acetate treatment; iodine treatment; hydrogen fluoride treatment; methanesulfonic acid treatment; thiophenol treatment

Recently, we examined the chemical properties of the sulfoxide of Cys(MBzl), as well as that of Cys(Bzl).[4] In this paper, we report on the behavior of the sulfoxide of Cys(Acm), another important cysteine derivative in peptide chemistry, especially under various deprotecting conditions.

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