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[Lab. of Biochemistry]

### **Synthesis and Pharmacological Evaluation in Mice of Halogenated Cannabidiol Derivatives.**

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Six halogenated derivatives of cannabidiol (CBD) substituted on the aromatic ring at the 3' and/or 5' position, 3'-chloro-, 3',5'-dichloro-, 3'-bromo-, 3',5'-dibromo-, 3'-iodo- and 3',5'-diiodo-CBD were synthesized and their pharmacological effects of barbiturate-induced sleep prolongation, anticonvulsant effects and locomotor activity were evaluated by intravenous (i.v.) injection in mice. These results indicate that monohalogenation of CBD leads to some modification of the pharmacological profile of CBD.

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[Lab. of Biochemistry]

### **Inhibition of Mouse Hepatic Glutathione S-Transferase by $\Delta^8$ -Tetrahydrocannabinol *p*-Quinone and Cannabidiol Hydroxy-quinone.**

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The effect of structurally related cannabinoid quinones,  $\Delta^8$ -tetrahydrocannabinol *p*-quinone ( $\Delta^8$ -THCPQ) and cannabidiol hydroxy-quinone (CBDHQ) on the activity of glutathione *S*-transferase (GST) in mouse hepatic microsomes (Ms) and 105,000  $\times$ g supernatant (SP) was studied. GST activities in Ms and SP from untreated (UT) and phenobarbital (PheB)-treated mice were measured using 1-chloro-2,4-dinitrobenzene (CDNB) and 1,2-dichloro-4-nitrobenzene (DCNB) as substrates. In all cases, both cannabinoid quinones concentration-dependently inhibited GST activities. In the case of the GST activity with DCNB, both cannabinoid quinones also concentration-dependently inhibited. In either case, CBDHQ inhibited the GST activity to a great extent than did  $\Delta^8$ -THCPQ. The kinetic parameters for CDBN, DCNB and glutathione (GSH) on the GST activity were determined using Lineweaver-Burk reciprocal plots. For CDBN and GSH in all GST activities, the type of inhibition by CBDHQ was competitive, but that by  $\Delta^8$ -THCPQ was non-competitive in those except for UT-Ms.  $\Delta^8$ -THCPQ also non-competitively inhibited in all GST activities for DCNB. In contrast, CBDHQ competitively inhibited GST activities for DCNB in the UT-Ms and UT-SP.

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[Lab. of Biochemistry]

### **A Cytochrome P450 Enzyme Responsible for the Carbon Monoxide Formation by Cannabidiol in Mouse Hepatic Microsomes.**

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The present study investigated major cytochrome P450 (P450) enzyme responsible for the carbon monoxide (CO) formation during hepatic microsomal mouse metabolism of cannabidiol (CBD). The present study demonstrated that a P450 enzyme belonging to CYP2A has some role for CBD-induced CO formation in mouse hepatic microsomes.

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[Lab. of Biochemistry]

### **Complete Nucleotide Sequence, Origin of Isoform and Functional Characterization of the Mouse Hepsin Gene.**

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Hepsin, a type-II membrane-associated serine protease, has been implicated in cell growth and development as well as possible initiation of blood coagulation. Here, we report on the complete nucleotide sequence, functional characterization of key structural features and the promoter of the mouse hepsin gene. The gene has a size of approximately 17 kb, and is composed of 12, 13, or 14 exons depending on alternative intron splicings - one in the 5'-UTR and the other two in the second intron. The latter two, which occur in approximately half of the hepsin transcripts, generate a hepsin mRNA species with an extra exon. The transcriptional initiation site was determined to be 636 bp upstream of the first ATG site in a cytidine-rich region. The 5'-flanking region of hepsin up to nucleotide 274 showed a substantial promoter activity in HepG2 cells. The basal promoter region contains potential binding sites for several transcription factors including SP1, AP2, C/EBP, LF-A1, and E box, which may be responsible for ubiquitous, but liver- and kidney-preferred tissue expression of the hepsin gene.