Biochemical Pharmacology, Vol. 60, pp. 955–961, 2000.

ISSN 0006-2952/00/$–see front matter
PII S0006-2952(00)00431-7

Effects of Unsaturated Side-chain Analogs of Tetrahydrocannabinol on Cytochromes P450

*Lester M. Bornheim**

Department of Cellular and Molecular Pharmacology and the Liver Center, University of California, San Francisco, CA 94143-0450, U.S.A.

ABSTRACT. The ability of unsaturated side-chain analogs of Δ^8-tetrahydrocannabinol (THC) to selectively inactivate mouse hepatic cytochromes P450 3A11 and 2C29 was examined. THC side-chain analogs were preincubated with mouse hepatic microsomes and NADPH for various times before dilution and determination of Δ^9-THC metabolism specific for P450s 3A11 and 2C29. THC-enyl analogs had little or no effect on P450 3A11 but inactivated P450 2C29 in a time-dependent manner, with approximately 50% inactivation observed after a 30-min preincubation. THC-ynyl analogs were less selective in their P450 inactivation but appeared to be more effective than their corresponding enyl analogs. THC-ynyl analogs inactivated P450s 3A11 and 2C29 in a time-dependent manner and could inactive 40–80% of their activities after a 30-min preincubation. The THC-ynyl analogs were nearly as effective as cannabidiol, a well-characterized inactivator of these mouse P450s. Despite their ability to inactivate P450 *in vitro*, neither the THC-enyl nor the THC-ynyl analogs were very effective after *in vivo* administration. Unsaturated side-chain THC analogs may be useful in the development of specific P450 inactivators. BIOCHEM PHARMACOL **60**;7:955–961, 2000. © 2000 Elsevier Science Inc.

KEY WORDS. cytochrome P450; P450 3A; P450 2C; cannabinoids; mechanism-based inactivation; tetrahydrocannabinol

Δ^9-THC† is the major psychoactive component of marijuana, and it exerts its effects primarily through binding at cannabinoid receptors, CB1 and CB2 [1]. In an ongoing attempt to design receptor subtype-selective ligands as agonists or antagonists, recent work has focused on alterations of the side-chain moiety of THC. Pharmacophore modeling of the cannabinoid receptor has led to a three-point model demonstrating the importance of the aliphatic side-chain of THC [2]. Structural variations of this moiety yielded analogs varying up to 3 orders of magnitude in affinity for the receptor and pharmacological potency [3]. In an attempt to introduce some rigidity and conformational restraint into the side-chain, several analogs with carbon–carbon double or triple bonds have been synthesized and evaluated for receptor affinity and pharmacological activity [4–8].

The inactivation of cytochromes P450 by terminal olefins and acetylenes is a mechanism-based process that has been well characterized and usually results in the alkylation of the prosthetic heme group [9–13]. P450 inactivation by internal olefins and acetylenes is far less common but has been reported, and typically results in P450 apoprotein modification [12–15]. Cannabinoids are metabolized extensively by P450s and dozens of metabolites have been identified [16]. The major metabolites found in most animal species are those oxidized at the 11- and 8-position (Fig. 1). In addition, minor metabolites hydroxylated at various positions in the side-chain as well as metabolites with shortened side-chains also have been reported [16, 17]. THC is oxidized in mice by P450 3A11 [18] primarily to 8β-hydroxy- and 8-keto-THC metabolites and by P450 2C29 [19] to 8α-hydroxy-THC, as determined by immunoinhibition and functional reconstitution studies with purified enzymes [20]. The formation of these metabolites was used as functional markers for P450s 3A and 2C in order to determine if oxidation at the double or triple bonds of several THC analogs could result in P450 inactivation.

MATERIALS AND METHODS

Chemicals

Δ^8-THC unsaturated analogs were synthesized as reported [5, 8] and include 2-ene, 3-ene, 4-ene, 2-yne, and 4-yne (Fig. 1). Δ^9-THC and CBD were supplied by the National Institute on Drug Abuse. Other chemicals and solvents were from Fisher Scientific and were of analytical or HPLC grade. Cannabinoids were dissolved in ethanol for the *in vitro* studies or suspended in Tween 80 for the *in vivo* studies as previously described [21].

* Correspondence. Department of Cellular and Molecular Pharmacology, Box 0450, University of California, San Francisco, CA 94143-0450. Tel. (415) 476-3872; FAX (415) 476-5292; E-mail: lmb@itsa.ucsf.edu

† *Abbreviations*: THC, tetrahydrocannabinol; CBD, cannabidiol; 2-ene, 3-(oct-2′-enyl)-Δ^8-THC; 3-ene, 3-(oct-3′-enyl)-Δ^8-THC; 4-ene, 3-(oct-4′-enyl)-Δ^8-THC; 2-yne, 3-(oct-2′-ynyl)-Δ^8-THC; and 4-yne, 3-(oct-4′-ynyl)-Δ^8-THC.

Received 17 September 1999; accepted 29 March 2000.

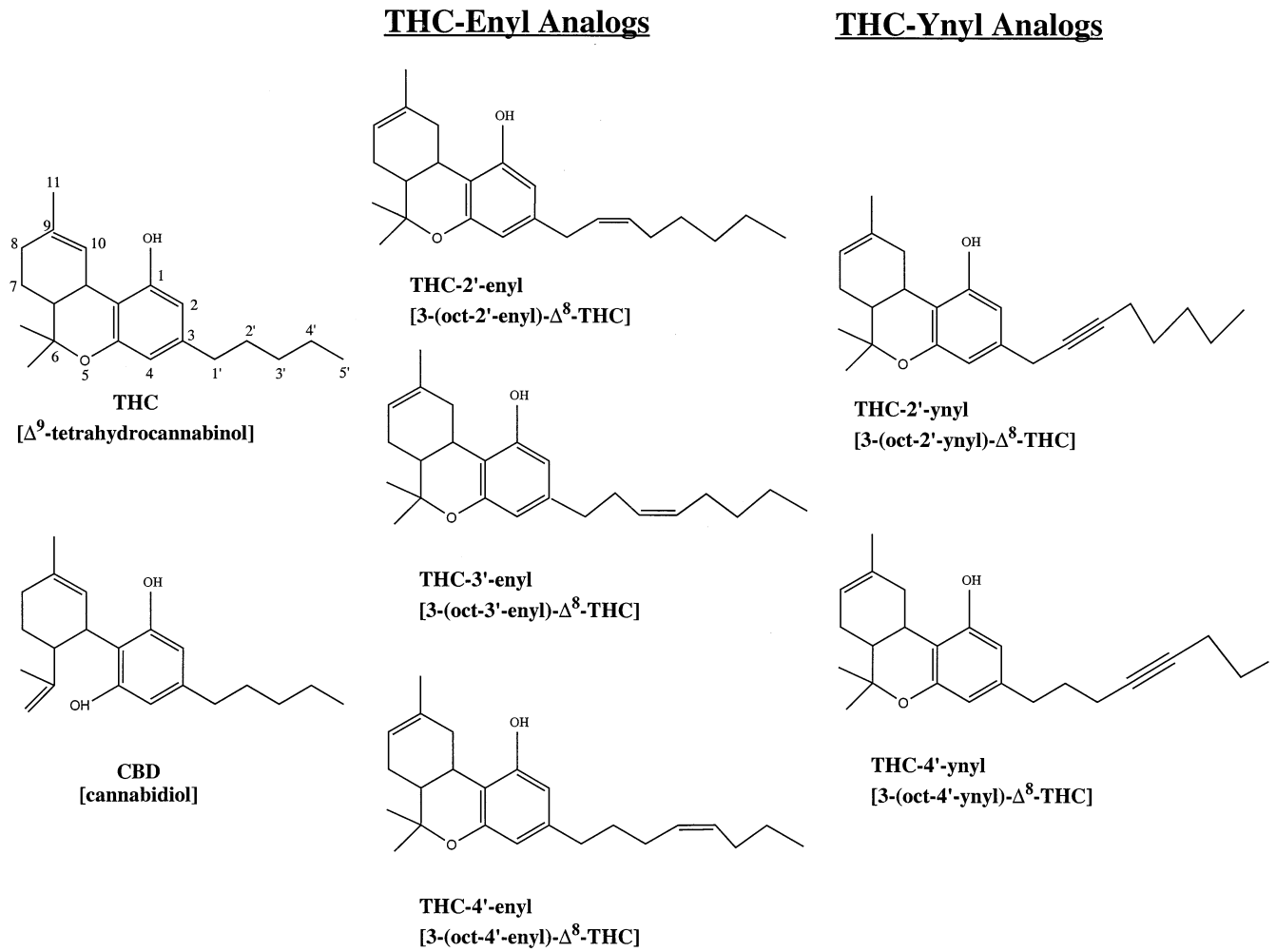

FIG. 1. Structures of Δ^9-THC, CBD, and Δ^8-THC side-chain analogs.

Animals

Male CF-1 mice (25–30 g; Charles River) were used for all studies.

In Vitro *Studies*

Mouse hepatic microsomes were prepared as described [22]. Microsomes (2 mg protein/mL) in 0.1 M phosphate buffer, pH 7.4, containing 1 mM diethylenetriaminepentaacetic acid were preincubated with ethanol (vehicle) or Δ^8-THC analog (130 μM in 3 μL ethanol) and NADPH (1 mM) in a total volume of 1 mL at 37° for 0, 7.5, 15, or 30 min. In parallel, CBD also was included in these studies as a known P450 inactivator. Aliquots (0.1 mL) were removed, diluted 10-fold with 0.1 M phosphate buffer, pH 7.4, containing Δ^9-THC (130 μM) and NADPH (1 mM), and incubated at 37° for an additional 10 min before the reaction was terminated by the addition of ethyl acetate (2 mL) and internal standard (dihydro-CBD). After vortexing and centrifugation, the organic layer was transferred to another tube, and the aqueous layer re-extracted again with ethyl acetate. The combined organic layers were pooled, dried under N_2, and chromatographed by reverse-phase HPLC as previously described [20] to determine THC metabolite formation. Control values (means ± SD of microsomal preparations from 3 different animals) for 8β-hydroxy-, 8α-hydroxy-, and 8-keto-THC formation were 0.19 ± 0.09, 2.90 ± 0.41, and 0.12 ± 0.04 nmol/mg/min, respectively.

In Vivo *Studies*

Mice were treated with Tween 80 vehicle or THC analog (60 mg/kg) for 2 hr. Animals were killed by cervical dislocation, livers were removed, and microsomes were prepared [22]. Hepatic microsomes (0.2 mg protein/mL) were incubated with THC (130 μM) and NADPH (1 mM) at 37° for 10 min before extraction with ethyl acetate (as described above) to determine THC metabolite formation.

RESULTS

THC-enyl and -ynyl analogs were preincubated with hepatic microsomes in order to determine their effect on subsequent THC metabolism. 8β-Hydroxy- and 8-keto-

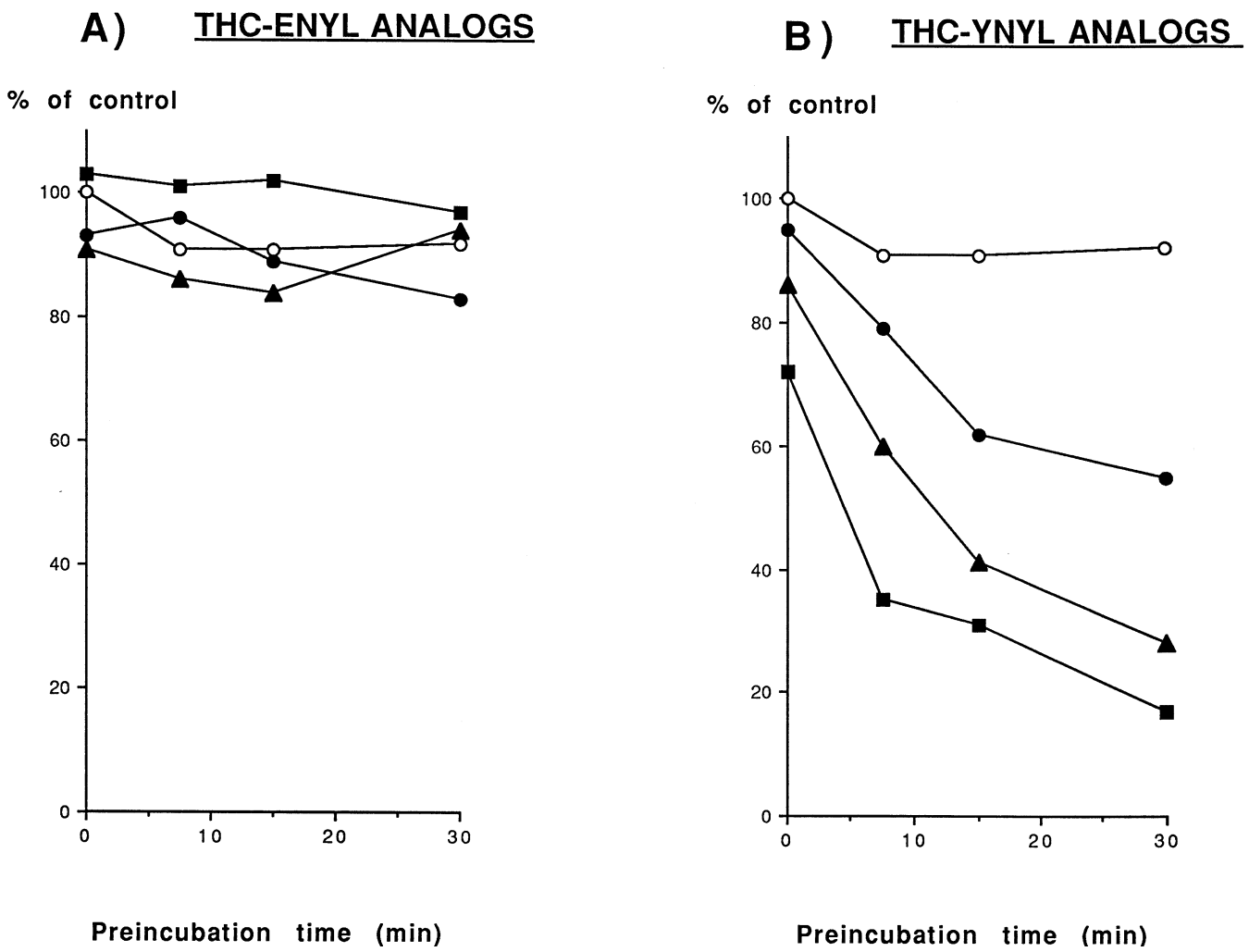

FIG. 2. Effects of THC analogs on P450 3A-mediated 8β-hydroxy-THC formation. (A) THC-enyl analogs (130 μM) were preincubated with hepatic microsomes (2 mg protein/mL) and NADPH (1 mM) at 37° for various times. Aliquots (0.1 mL) were diluted 10-fold with 0.1 M phosphate buffer, pH 7.4, containing THC (130 μM) and NADPH (1 mM), and incubated at 37° for an additional 10 min before the reaction was terminated by the addition of ethyl acetate. 8β-Hydroxy-THC formation was determined as described (see Materials and Methods). The effects of no inhibitor (open circles), 2-ene (solid circles), 3-ene (solid squares), or 4-ene (solid triangles) THC analogs on THC metabolite formation represent the average of two different experiments. Control (100%) 8β-hydroxy-THC formation was 0.33 nmol/mg/min. (B) THC-ynyl analogs or CBD were preincubated with hepatic microsomes as described in panel A before 8β-hydroxy-THC formation was determined. The effects of no inhibitor (open circles), 2-yne (solid circles), 4-yne (solid squares), THC analogs or CBD (solid triangles) on THC metabolite formation represent the average of two different experiments.

THC formation (catalyzed in mice by P450 3A11) and 8α-hydroxy-THC formation (catalyzed by P450 2C29) were used as functional markers to assess their respective P450 inactivation.

THC-enyl analogs had little effect on P450 3A11 as determined by 8β-hydroxy- (Fig. 2A) or 8-keto-THC formation (Fig. 3A). In contrast, THC-enyl analogs inactivated P450 2C29-mediated 8α-hydroxy-THC formation in a time-dependent manner (Fig. 4A), with approximately 50% inactivation observed after a 30-min preincubation.

THC-ynyl analogs were less selective in their P450 inactivation but appeared to be more effective than their corresponding enyl analogs. THC-ynyl analogs inactivated P450 3A11 in a time-dependent manner (Figs. 2B and 3B) and could inactivate 40–80% of its activity after a 30-min preincubation. The effect of THC-2-ynyl on 8-keto-THC formation could not be determined because of the co-elution of a presumed THC-2-ynyl metabolite with 8-keto-THC.

As observed with the THC-enyl analogs, the THC-ynyl analogs also inactivated P450 2C29 in a time-dependent manner (Fig. 4B) but were somewhat more effective, inactivating up to 80% of its activity after a 30-min preincubation. In fact, the THC-ynyl analogs were nearly as effective as CBD, a well-characterized inactivator of mouse P450s 3A11 and 2C29 [20, 23, 24].

Despite their ability to inactivate P450 *in vitro*, neither the THC-enyl nor the -ynyl analogs were very effective after *in vivo* administration (Fig. 5). In contrast, CBD was a very effective inactivator of P450s 3A11 and 2C29 after *in*

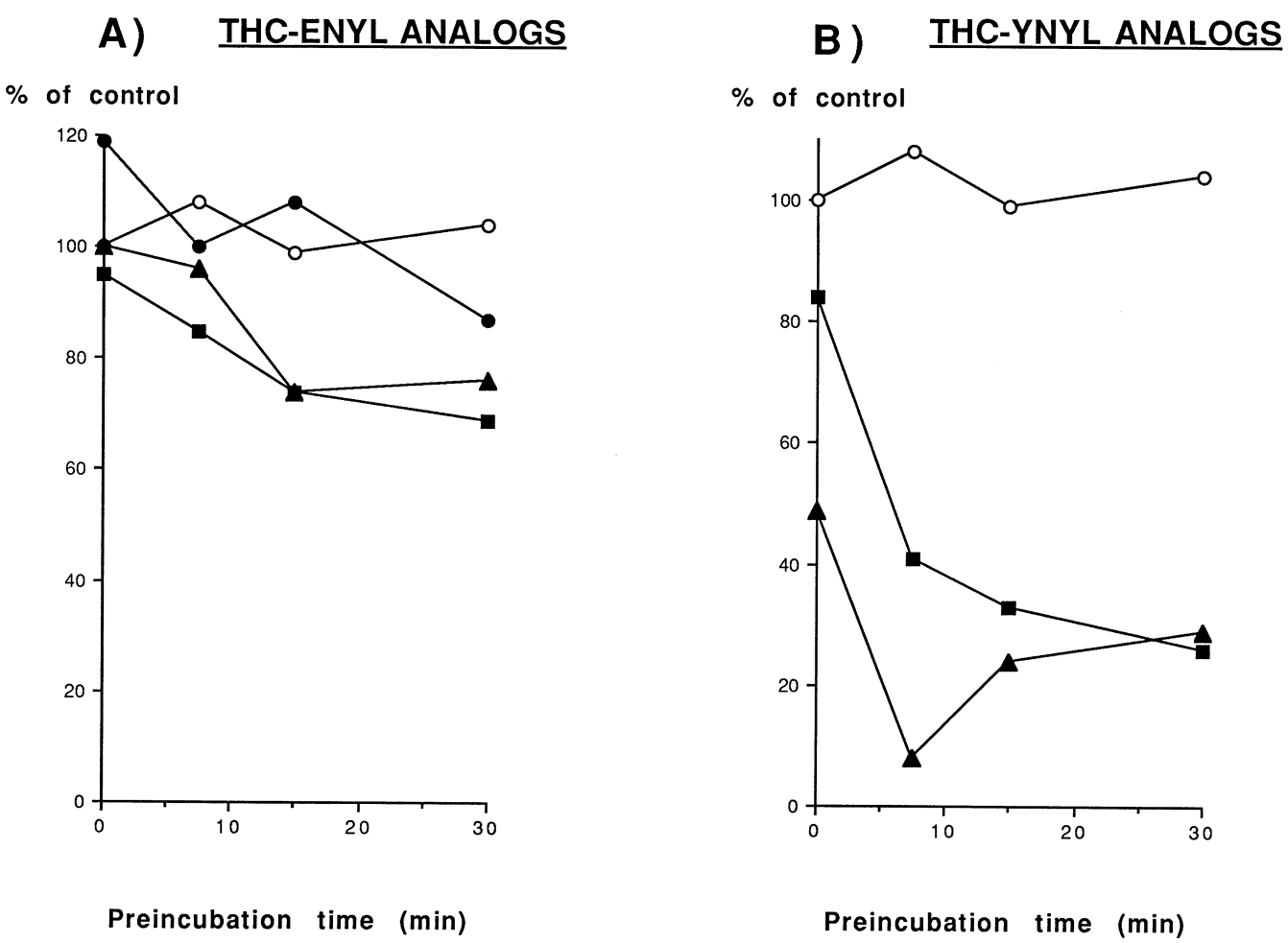

FIG. 3. Effects of THC analogs on P450 3A-mediated 8-keto-THC formation. (A) THC-enyl analogs (130 μM) were preincubated with hepatic microsomes as described in Fig. 2A. 8-Keto-THC formation was determined as described (see Materials and Methods). Symbols used are identical to those in Fig. 2A. Control (100%) 8-keto-THC formation was 0.27 nmol/mg/min. Values represent the average of two experiments. (B) THC-ynyl analogs or CBD were preincubated with hepatic microsomes as described in panel A before 8-keto-THC formation was determined. Symbols used are identical to those in Fig. 2B. The effect of THC-2-ynyl on 8-keto-THC formation could not be determined because of the co-elution of a presumed THC-2-ynyl metabolite. Values represent the average of two experiments.

vivo administration. As reported previously [20, 23], despite the observed P450 3A11 inactivation by CBD treatment as evidenced by decreased 8-keto-THC formation and other P450 3A-catalyzed activities (testosterone 6β-hydroxylase, erythromycin *N*-demethylase), 8β-hydroxy-THC formation was paradoxically increased after CBD treatment.

DISCUSSION

There is much interest in developing specific P450 inactivators to treat a variety of diseases or influence the pharmacokinetics of therapeutic drugs [14, 25, 26]. We have characterized the inactivation of hepatic microsomal P450s after preincubation with several THC analogs containing double or triple bonds within their side-chain moiety to determine their selectivity for P450s 3A and 2C. Competitive inhibition by the analogs was minimized by dilution of microsomal incubates after preincubation with the inhibitors, and before the determination of THC hydroxylase activity. In fact, competitive inhibition by the analogs (as determined by reduced activity at preincubation time = 0 min) was <20% of control activities, and therefore the observed time-dependent decreases in enzyme activity largely represent enzyme inactivation.

THC-enyl analogs could selectively inactivate P450 2C29 *in vitro*, although at a slower rate than CBD, a known P450 inactivator present in marijuana [24, 27]. In contrast to CBD, THC-enyl analogs failed to inactivate P450 3A11, indicating their relative selectivity for members of the P450 2C subfamily. In contrast to the selective P450 2C inactivation by the THC-enyl analogs, THC-ynyl analogs inactivated both P450s 3A11 and 2C29 *in vitro*, comparably to CBD. This is consistent with previous reports indicating that acetylenic compounds are more efficient P450 inactivators than olefins [13].

Neither THC-enyl nor -ynyl analogs were effective P450 inactivators *in vivo*. These analogs may possess poor bioavailability since a similar dissociation between their *in*

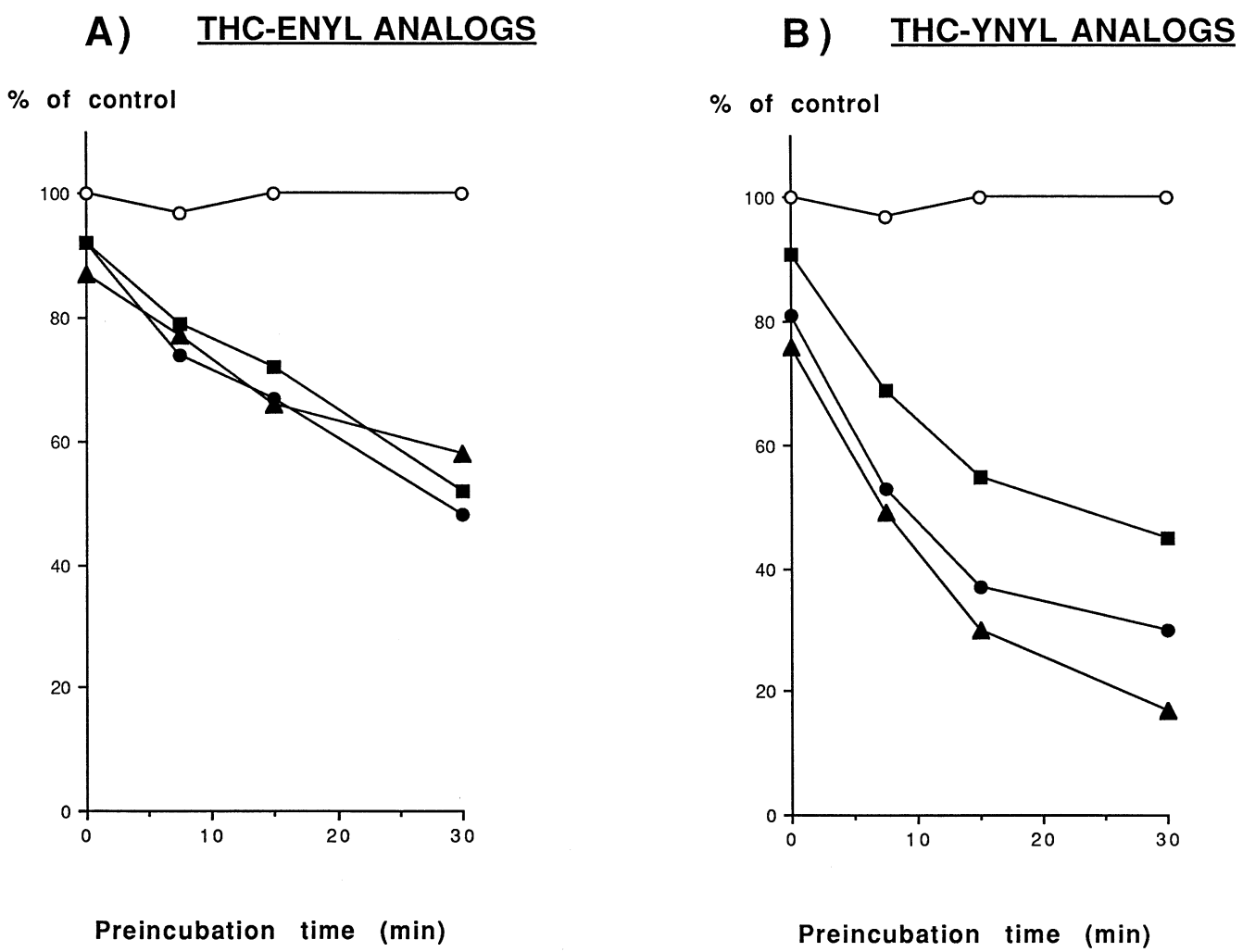

FIG. 4. Effects of THC analogs on P450 2C-mediated 8α-hydroxy-THC formation. (A) THC-enyl analogs (130 μM) were preincubated with hepatic microsomes as described in Fig. 2A. 8α-Hydroxy-THC formation was determined as described (see Materials and Methods). Symbols used are identical to those in Fig. 2A. Control (100%) 8α-hydroxy-THC formation was 3.38 nmol/mg/min. Values represent the average of two experiments. (B) THC-ynyl analogs or CBD were preincubated with hepatic microsomes as described in panel A before 8α-hydroxy-THC formation was determined. Symbols used are identical to those in Fig. 2B. Values represent the average of two experiments.

vitro binding affinities to the CB1 receptor and their pharmacological potencies has been reported previously [5, 28]. However, it is also possible that they were actually accessible to the receptor but acted as antagonists rather than agonists [8].

Although P450 inactivation by compounds with terminal olefinic and acetylenic bonds has been well described and involves a reactive ketene intermediate, inactivation after oxidation at internal unsaturated positions is far less common [11–13]. $P450_{scc}$ was inactivated by a pregnenolone analog containing an internal acetylenic group at the C_{20} position presumably through an oxirene intermediate, although another analog containing an additional terminal acetylenic group was an even greater inactivator [14]. Mid-chain acetylenes were also shown to be inactivators of P450 2B4, whereas only terminal acetylenes inactivated P450s 4A or plant lauric acid ω-hydroxylases [15].

P450 inactivation with heme alkylation only occurs after oxidative attack at terminal bonds, whereas oxidation at internal bonds results in P450 inactivation without any detectable heme adducts [12, 13]. Thus, it is conceivable that the P450 inactivation by the THC analogs used in this study resulted from protein alkylation rather than heme adduct formation.

Although several P450 3A-selective inhibitors/inactivators are known, few compounds selective for P450 2C inhibition have been reported. Sulfaphenazole is a selective inhibitor of human P450 2C9 [29, 30] and exerts its inhibition by a tight interaction between its aniline function and the P450 heme iron [31]. This interaction is selective for P450 2C9, and does not occur with human P450s 2C8, 2C18, or 2C19.

Human P450s 2C8, 2C9, 2C18, and 2C19 ring-hydroxylate THC at the 8- and 11-carbon positions at various rates [32, 33]. Although human liver microsomes support THC side-chain hydroxylation with metabolites identified at

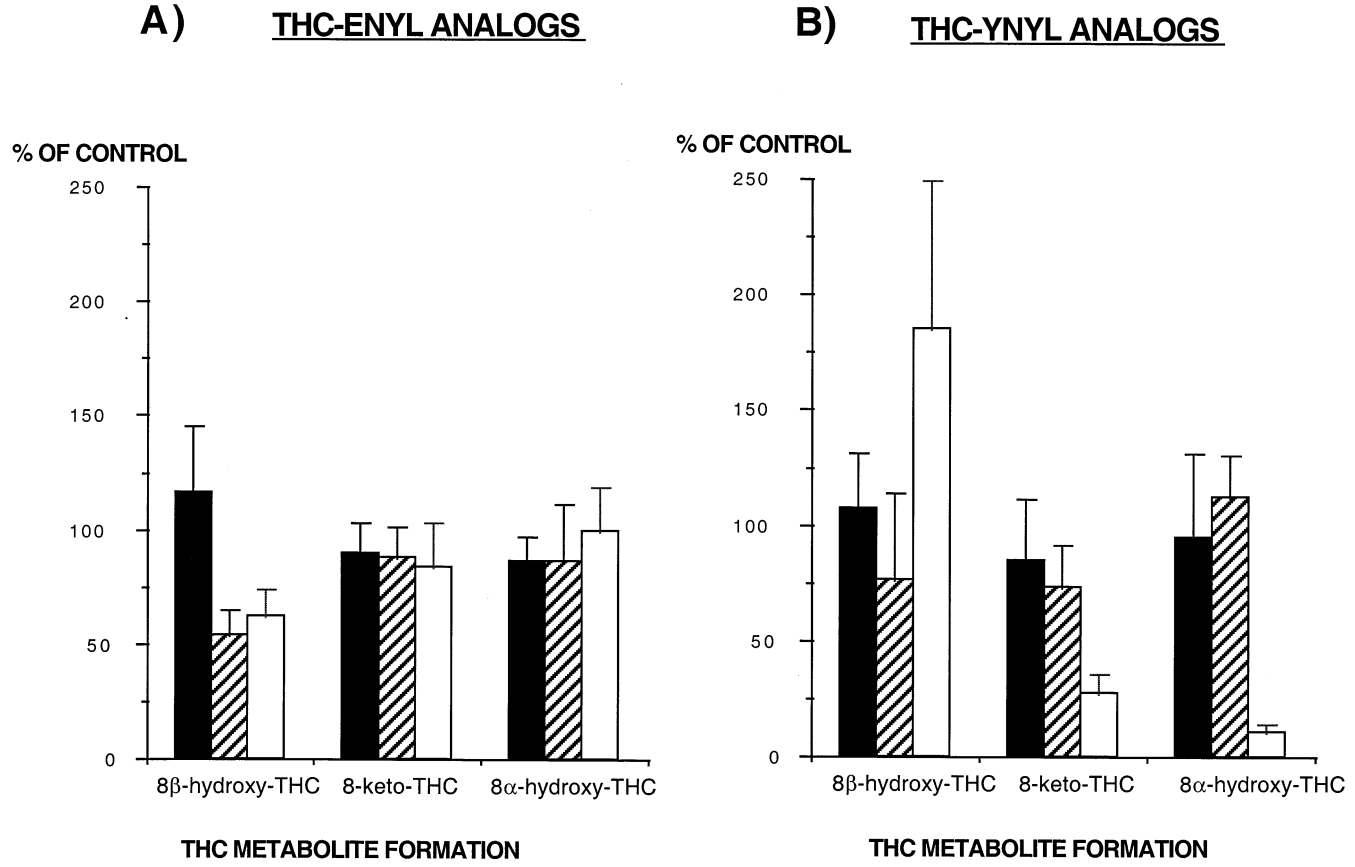

FIG. 5. ***In vivo*** **effects of THC analogs on P450s 3A- and 2C-mediated THC metabolite formation. (A) Mice were treated with THC-enyl analogs (60 mg/kg for 2 hr) before hepatic microsomes were prepared and assayed for THC metabolite formation as described (see Materials and Methods). The effects of 2-ene (solid bars), 3-ene (striped bars), or 4-ene (open bars) THC analogs on THC metabolite formation represent the means ± SD of microsomal preparations from 3 different animals. Control (100%) values for 8β-hydroxy-, 8α-hydroxy-, and 8-keto-THC formation were 0.19 ± 0.09, 2.90 ± 0.41, and 0.12 ± 0.04 nmol/mg/min, respectively. (B) Mice were treated with THC-ynyl analogs or CBD (60 mg/kg for 2 hr) before hepatic microsomes were prepared and assayed for THC metabolite formation as described (see Materials and Methods). The effects of 2-yne (solid bars), 4-yne (striped bars), THC analogs or CBD (open bars) on THC metabolite formation represent the means ± SD of microsomal preparations from 3 different animals.**

each carbon position of the side-chain [16, 17, 34, 35], the P450s mediating such THC side-chain hydroxylations have not been identified. Thus, the THC analogs with unsaturated side-chains used in this study may be useful in the development of specific inactivators of the various members of the human P450 2C subfamily or possibly other P450s that may oxidize these analogs at their unsaturated bonds. In addition, the possible toxicological consequences of P450 inactivation may need to be considered should these analogs become therapeutically useful.

These studies were supported by National Institute of Health Grant DA-04265. Raj K. Razdan, Organix, Inc. (DA-05488) is gratefully acknowledged for supplying the THC analogs used in these studies, as is the Liver Center Core Facility on Spectrophotometry (DK-26743).

References

1. Pertwee R, Pharmacology of cannabinoid CB1 and CB2 receptors. *Pharmacol Ther* **74:** 129–180, 1997.
2. Martin B, Thomas B and Razdan R, Structural requirements for cannnabinoid receptor probes. In: *Cannabinoid Receptors* (Ed. Pertwee R), pp. 35–86. Academic Press, London, 1995.
3. Makriyannis A and Rapaka RS, The molecular basis of cannabinoid activity. *Life Sci* **47:** 2173–2184, 1990.
4. Razdan RK, Dalzell HC, Herlihy P and Howes JF, Hashish: Unsaturated side-chain analogues of Δ^8-tetrahydrocannabinol with potent biological activity. *J Med Chem* **19:** 1328–1330, 1976.
5. Ryan W, Singer M, Razdan RK, Compton DR and Martin BR, A novel class of potent tetrahydrocannabinols (THCS): 2′-yne-Δ^8- and Δ^9-THCS. *Life Sci* **56:** 2013–2020, 1995.
6. Busch-Petersen J, Hill WA, Fan P, Khanolkar A, Xie X-Q, Tius MA and Makriyannis A, Unsaturated side chain β-11-hydroxy-hexahydrocannabinol analogs. *J Med Chem* **39:** 3790–3796, 1996.
7. Papahatjis DP, Kourouli T, Abadji V, Goutopoulos A and Makriyannis A, Pharmacophoric requirements for cannabinoid side chains: Multiple bond and C1′-substituted Δ^8-tetrahydrocannabinols. *J Med Chem* **41:** 1195–1200, 1998.
8. Griffin G, Wray EJ, Rorrer WK, Crocker PJ, Ryan WJ, Saha B, Razdan RK, Martin BR and Abood ME, An investigation into the structural determinants of cannabinoid receptor ligand efficacy. *Br J Pharmacol* **126:** 1575–1584, 1999.

9. Ortiz de Montellano P and Kunze K, Self-catalyzed inactivation of hepatic cytochrome P-450 by ethynyl substrates. *J Biol Chem* **255:** 5578–5585, 1980.
10. White I, Structure-activity relationships in the destruction of cytochrome P-450 mediated by certain ethynyl-substituted compounds in rats. *Biochem Pharmacol* **29:** 3253–3255, 1980.
11. Ortiz de Montellano PR, Alkenes and alkynes. In: *Bioactivation of Foreign Compounds* (Ed. Anders M), pp. 121–155. Academic Press, New York, 1985.
12. Ortiz de Montellano PR and Reich NO, Inhibition of cytochrome P-450 enzymes. In: *Cytochrome P-450: Structure, Mechanism, and Biochemistry* (Ed. Ortiz de Montellano PR), pp. 273–314. Plenum Press, New York, 1986.
13. Ortiz de Montellano PR and Correia MA, Inhibition of cytochrome P450 enzymes. In: *Cytochrome P450: Structure, Mechanism, and Biochemistry* (Ed. Ortiz de Montellano PR), 2nd Edn, pp. 305–364. Plenum Press, New York, 1995.
14. Nagahisa A, Spencer RW and Orme-Johnson WH, Acetylenic mechanism-based inhibitors of cholesterol side chain cleavage by cytochrome P-450$_{scc}$. *J Biol Chem* **258:** 6721–6723, 1983.
15. Helvig C, Alayrac C, Mioskowski C, Koop D, Poullain D, Durst F and Salaün J-P, Suicide inactivation of cytochrome P450 by midchain and terminal acetylenes. A mechanistic study of inactivation of a plant lauric acid ω-hydroxylase. *J Biol Chem* **272:** 414–421, 1997.
16. Harvey DJ and Paton WDM, Metabolism of the cannabinoids. In: *Reviews in Biochemical Toxicology* (Eds. Hodgson E, Bend JR and Philpot RM), Vol. 6, pp. 221–264. Elsevier, New York, 1986.
17. Yamamoto I, Narimatsu S, Watanabe K, Shimonishi T, Yoshimura H and Nagano T, Human liver microsomal oxidation of Δ^8-tetrahydrocannabinol. *Chem Pharm Bull (Tokyo)* **31:** 1784–1787, 1983.
18. Yanagimoto T, Itoh S, Muller-Enoch D and Kamataki T, Mouse liver cytochrome P-450 (P-450IIIAM1): Its cDNA cloning and inducibility by dexamethasone. *Biochim Biophys Acta* **1130:** 329–332, 1992.
19. Matsunaga T, Watanabe K, Yamamoto I, Negishi M, Gonzalez F and Yoshimura H, cDNA cloning and sequence of CYP2C29 encoding P-450 MUT-2, a microsomal aldehyde oxygenase. *Biochim Biophys Acta* **1184:** 299–301, 1994.
20. Bornheim LM and Correia MA, Purification and characterization of the major hepatic cannabinoid hydroxylase in the mouse: A possible member of the cytochrome P-450IIC subfamily. *Mol Pharmacol* **40:** 228–234, 1991.
21. Bornheim LM, Borys HK and Karler R, Effect of cannabidiol on cytochrome P-450 and hexobarbital sleep time. *Biochem Pharmacol* **30:** 503–507, 1981.
22. Bornheim LM and Correia MA, Effect of cannabidiol on cytochrome P-450 isozymes. *Biochem Pharmacol* **38:** 2789–2794, 1989.
23. Bornheim LM and Correia MA, Selective inactivation of mouse liver cytochrome P-450IIIA by cannabidiol. *Mol Pharmacol* **38:** 319–326, 1990.
24. Bornheim LM, Everhart ET and Correia MA, Characterization of cannabidiol-mediated cytochrome P-450 inactivation. *Biochem Pharmacol* **45:** 1323–1331, 1993.
25. Correia MA and Ortiz de Montellano PR, Inhibitors of cytochrome P450 and possibilities for their therapeutic application. In: *Frontiers in Biotransformation* (Ed. Ruckpaul K), pp. 74–146. Akademie-Verlag, Berlin, 1993.
26. Ling Y-Z, Li J-S, Liu Y, Kato K, Klus GT and Brodie A, 17-Imidazolyl, pyrazolyl, and isoxazolyl androstene derivatives. Novel steroidal inhibitors of human cytochrome $C_{17,20}$-lyase ($P450_{17\alpha}$). *J Med Chem* **40:** 3297–3304, 1997.
27. Watanabe K, Arai M, Narimatsu S, Yamamoto I and Yoshimura H, Self-catalyzed inactivation of cytochrome P-450 during microsomal metabolism of cannabidiol. *Biochem Pharmacol* **36:** 3371–3377, 1987.
28. Martin BR, Jefferson R, Winckler R, Wiley JL, Huffman JW, Crocker PJ, Saha B and Razdan RK, Manipulation of the tetrahydrocannabinol side chain delineates agonists, partial agonists, and antagonists. *J Pharmacol Exp Ther* **290:** 1065–1079, 1999.
29. Correia MA, Rat and human liver cytochromes P450: Substrate and inhibitor specificities and functional markers. In: *Cytochrome P450: Structure, Mechanism, and Biochemistry* (Ed. Ortiz de Montellano PR), 2nd Edn, pp. 607–630. Plenum Press, New York, 1995.
30. Newton D, Wang R and Lu A, Cytochrome P450 inhibitors: Evaluation of specificities in the *in vitro* metabolism of therapeutic agents by human liver microsomes. *Drug Metab Dispos* **23:** 154–158, 1995.
31. Mancy A, Dijols S, Poli S, Guengerich P and Mansuy D, Interaction of sulfaphenazole derivatives with human liver cytochromes P450 2C: Molecular origin of the specific inhibitory effects of sulfaphenazole on CYP 2C9 and consequences for the substrate binding site topology of CYP 2C9. *Biochemistry* **35:** 16205–16212, 1996.
32. Bornheim LM, Lasker JM and Raucy JL, Human hepatic microsomal metabolism of Δ^1-tetrahydrocannabinol. *Drug Metab Dispos* **2:** 241–246, 1992.
33. Richardson TH, Jung F, Griffin KJ, Wester M, Raucy JL, Kemper B, Bornheim LM, Hassett C, Omiecinski CJ and Johnson EF, A universal approach to the expression of human and rabbit cytochrome P450s of the 2C subfamily in *Escherichia coli*. *Arch Biochem Biophys* **323:** 87–96, 1995.
34. Harvey DJ, Further studies on the oxidative cleavage of the pentyl side-chain of cannabinoids: Identification of new biotransformation pathways in the metabolism of 3′-hydroxy-delta-9-tetrahydrocannabinol in the mouse. *Xenobiotica* **19:** 1437–1447, 1989.
35. Yamamoto I, Watanabe K, Narimatsu S and Yoshimura H, Recent advances in the metabolism of cannabinoids. *Int J Biochem Cell Biol* **27:** 741–746, 1995.