

CHARACTERIZATION OF EPRINOMECTIN *N*-DEACETYLASE IN RATS

ZHAOPIE ZENG AND NICHOLAS W. ANDREW

Merck Research Laboratories, Merck & Co., Inc., Three Bridges, New Jersey

(Received April 16, 1998; accepted October 12, 1998)

This paper is available online at <http://www.dmd.org>

ABSTRACT:

The enzyme system responsible for the *N*-deacetylation of eprinomectin in rats was characterized. Tissue and subcellular studies showed that the hydrolysis activity was localized mainly in liver microsomes. Apparent K_M and V_{max} values calculated from Lineweaver-Burk plots were 53 μ M and 0.81 nmol/mg/min for male rats and 70 μ M and 4.99 nmol/mg/min for female rats, respectively. Pretreatment of male rats with dexamethasone, phenobarbital, and pregnenolone 16 α -carbonitrile increased the activity by more than 3-fold. Paraoxon and bis-4-nitrophenylphosphate strongly inhibited the deacetylase activity at concentrations as low as 1 μ M. The hydrolysis activity also was inhibited by SKF525, but less effectively. Eserine strongly inhibited the activity at 1×10^{-4} M. HgCl₂ decreased the activity to about 40% at a concentration of

1×10^{-4} M. FeCl₃, CaCl₂, MgCl₂, and EDTA had little effect on the hydrolysis of eprinomectin, whereas NaF slightly increased the activity to 118%. Thus, the inhibition study suggested that eprinomectin deacetylase resembled "B" type carboxylesterase/amidases. The hydrolysis activity of eprinomectin and isocarboxazid, a specific substrate of RL2 [Hosokawa, M, Maki T and Satoh T (1987) *Mol Pharmacol* 31:579-584], by liver microsomes from rats treated with various cytochrome P-450 inducers correlated well ($r = 0.92$). Also, elution profiles of esterase by gel filtration and ion exchange chromatography demonstrated that the active protein(s) for eprinomectin and isocarboxazid hydrolysis coeluted. Thus, RL2 or an enzyme system similar to RL2 is responsible for the *N*-deacetylation of eprinomectin.

Eprinomectin (4''-epiacetylamino-4''-deoxyavermectin B₁, or L-653,648), a member of a new class of avermectins, the aminoavermectin, has been approved in some countries for the control of parasites in beef and dairy cattle (Cvetovich et al., 1994; Shoop et al., 1996). Eprinomectin is a derivative of avermectin B₁ with a chemically modified terminal oleandrose moiety (Fig. 1). Eprinomectin consists of a mixture of two homologous compounds with a major component, 4''-epiacetylamino-4''-deoxyavermectin B_{1a} (AAB_{1a})¹ (>90% of the mixture), and a minor component, AAB_{1b} (<10% of the mixture), differing by a single methylene group.

In a previous paper (Zeng et al., 1996), we described the distribution, excretion, and metabolism of eprinomectin in the Sprague-Dawley VAF rats after oral administration. Overall, the majority of the dose was excreted unmetabolized in the feces. However, *N*-deacetylation of eprinomectin, the primary route of metabolism, was sex-dependent, i.e., the drug was metabolized more extensively in female rats than in male rats. The sex difference in *N*-deacetylation was also demonstrated in vitro. In this study, characteristics of the enzyme system responsible for the *N*-deacetylation of eprinomectin were examined.

This work was presented in part at the Seventh North American International Society for the Study of Xenobiotics, San Diego, CA, Oct. 20-24, 1996, and published in abstract form.

¹ Abbreviations used are: AAB_{1a}, 4''-epiacetylamino-4''-deoxyavermectin B_{1a}; AAB_{1b}, 4''-epiacetylamino-4''-deoxyavermectin B_{1b}; PCN, pregnenolone-16 α -carbonitrile; Dex, dexamethasone; PB, phenobarbital; PNPA, *p*-nitrophenylacetate; BPNP, bis-4-nitrophenylphosphate; 3MC: 3-methylcholanthrene.

Send reprint requests to: Merck Limited, 2100 Ronson Road, Mailcode-NB, Iselin, NJ 08830-3077. E-mail: zhaopie.zeng@merck.com

Materials and Methods

Materials. [5-³H]AAB_{1a} (20.74 mCi/mg and $\geq 98.3\%$ pure) was synthesized by the Labeled Compound Synthesis Group, Drug Metabolism, Merck Research Laboratories. Unlabeled eprinomectin (AAB_{1a}/AAB_{1b}: 92.0/8.0, 97.5% pure) and aminoavermectin (4''-epiamino-4''-deoxyavermectin B₁; components AB_{1a}/AB_{1b}: 91.3/8.7; 95.6% pure) were obtained from the Chemical Data Department, Merck Research Laboratories. Substrates for incubations were prepared by mixing [5-³H]AAB_{1a} with unlabeled eprinomectin; thus, only the AAB_{1a} component was radiolabeled. Isocarboxazid was obtained from the Chemical Data Department, Merck Research Laboratories. All solvents used were of high-performance liquid chromatography (HPLC) grade or equivalent. Scintillation fluids Monophase and Insta-Gel XF were obtained from Packard (Downers Grove, IL). Other chemicals were purchased from Aldrich (Milwaukee, WI). Liver microsomes from chemically treated rats and control microsomes were purchased from Xenotech LLC (Kansas City, KS).

Tissue Preparation and *N*-Deacetylation of Eprinomectin by Selective Tissues. Male or female Sprague-Dawley rats (2 months old) were euthanized using CO₂. Pooled livers, kidneys, spleens, small intestine, brains, muscle, lungs, or hearts were washed with ice-cold 50 mM Tris buffer containing 1.15% KCl (pH 7.5) and then homogenized in a cooled blender with 4 volumes of the same buffer. Liver cytosol was prepared by centrifuging the liver homogenate at 10,000g for 20 min and centrifuging the resulting supernatant at 105,000g for 60 min. Plasma was obtained by centrifuging whole blood. [³H]Eprinomectin (10 μ M) was incubated with 400 μ l of tissue homogenates, liver cytosol, whole blood, and plasma from male or female rats for 4 h at 37°C. The reactions were stopped by adding 400 μ l of unlabeled eprinomectin in methanol. The incubation mixtures were centrifuged and the supernatants were analyzed by HPLC.

Liver Microsomal Incubation with Eprinomectin. Microsomal preparation from untreated rats has been described elsewhere (Zeng et al., 1996). The microsomal samples were diluted with either 0.1 M Tris buffer or 0.1 M potassium phosphate (pH 8) to 0.5 mg/ml protein. After a 45-min incubation with the substrate (25 μ M) at 37°C, the reactions were stopped by mixing with an equal volume of methanol solution containing a mixture of unlabeled

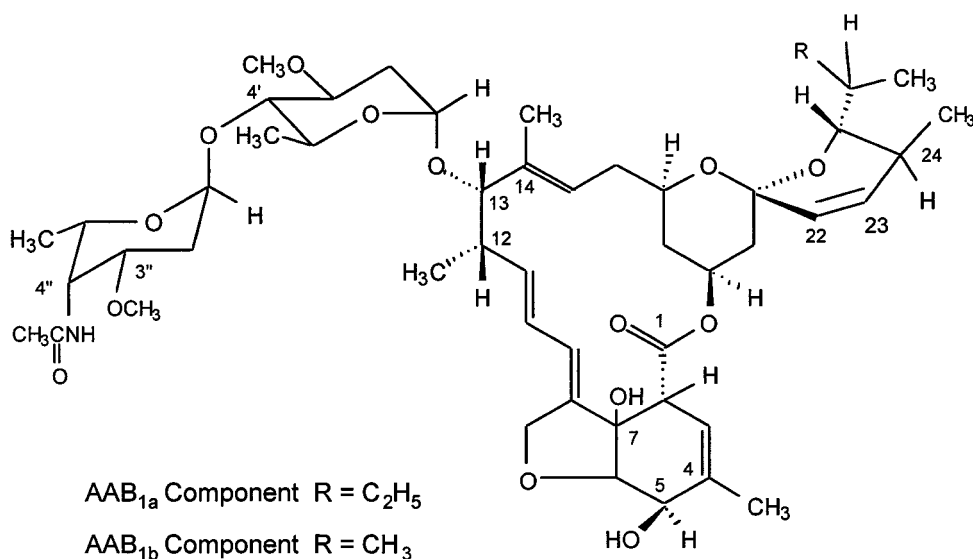


FIG. 1. Structure of eprinomectin.

eprinomectin and aminoavermectin. The incubation mixtures were centrifuged and the supernatants were analyzed by HPLC. Incubations with and without an NADPH-generating system (10 mM glucose 6-phosphate, 1 mM NADP⁺, and 4 units glucose 6-phosphate dehydrogenase) were compared.

Kinetic studies were carried out at eprinomectin concentrations ranging from 8 to 100 μ M. Rate of *N*-deacetylation of eprinomectin was measured by formation of aminoavermectin (Zeng et al., 1996). For inhibition studies with chemical inhibitors, female liver microsomes were used because they showed higher activity in the *N*-deacetylation of eprinomectin. Microsomes were preincubated with inhibitors at 37°C for 10 min before initiating the reaction by the addition of eprinomectin.

The microsomal activity on *N*-deacetylation of eprinomectin was examined as a function of pH in increments of 0.5 pH units using acetate buffer (50 mM) for pH range 4.0 to 6.5, Tris buffer (50 mM) for pH range 7.0 to 9.0, phosphate buffer (100 mM) for pH range 6.0 to 9.0, and carbonate buffer (100 mM) for pH 9.0 to 10.0. In controls, microsomes were omitted from the incubation medium to monitor the rate of nonenzymatic hydrolysis.

HPLC. After incubation, metabolite profiles were determined by reverse-phase HPLC analysis. Chromatography was performed on a Zorbax ODS 4.6-mm \times 250-mm column using a Shimadzu dual-pump gradient system with a mobile phase of CH₃CN/CH₃OH/H₂O (v/v) containing 5 mM ammonium acetate (System I: 46.2/30.8/23 for 35 min, 40–45 min, 46.2/30.8/23 to 60/40/0 in 5 min, 60/40/0 for 5 min, 60/40/0 to 46.2/30.8/23 in 10 min; System II: 51.6/34.4/14 for 19 min, 51.6/34.4/14 to 60/40/0 in 2 min, 60/40/0 for 6 min, 60/40/0 to 51.6/34.4/14 in 3 min). The flow rate was 1.0 ml/min, and the eluate was monitored at 245 nm using a flow-through UV detector. One-minute fractions of the column eluate were collected into miniscintillation vials and mixed with scintillation cocktail. The radioactivity in these samples was determined by scintillation spectrometry.

Determination of Hydrolysis Rate of *p*-Nitrophenylacetate (PNPA), Butanilicaine, Isocarboxazid, and Palmitoyl-CoA by Liver Microsomes. For hydrolysis of PNPA (1 mM, 25°C), butanilicaine (1 mM, 37°C), and palmitoyl-CoA (50 μ M, 37°C) from incubation with liver microsomes for 1, 45, and 30 min, respectively, the hydrolysis products were measured directly using a spectrophotometer at wavelengths of 400, 285, and 412 nm, respectively (Krisch, 1966; Berge, 1979; Heymann et al., 1981). The protein concentrations of the liver microsomes used were 25, 50, and 150 μ g/ml in 0.1 M phosphate buffer (pH 7.4), respectively. The hydrolysis of isocarboxazid was assayed in 0.1 M phosphate buffer (pH 7.4) by colorimetric determination of the released benzylhydrazine according to the method of Satoh and Moroi (1973).

Partial Purification of Esterase from Liver Microsomes. The procedure of Hosokawa et al. (1987) was followed with some modification. Liver microsomes from rats treated with phenobarbital (PB) were diluted to 2.5

mg/ml with 100 mM Tris-HCl buffer (pH 8.0). After addition of 1% saponin, the solution was stirred at 4°C for 60 min followed by ammonium sulfate fractionation. The 40 to 70% precipitate was suspended in 0.1 mM Tris-HCl buffer and desalted using a Centriprep 30 filtration system (MWCO 30,000; Amicon, Beverly, MA). The solution was subjected to gel filtration on a Superdex 200HR 10/30 column using 10 mM Tris-HCl buffer (pH 8.0) containing 100 mM NaCl, and the fractions were checked for hydrolysis activity with PNPA and eprinomectin.

Active fractions were pooled and desalted using a Centriprep 30 filtration system and chromatographed on a Mono Q anion exchange column (Pharmacia Biotech, Piscataway, NJ). The column was pre-equilibrated with 10 mM Tris-HCl buffer (pH 8.0) and eluted with a linear NaCl gradient in the buffer (0–250 mM NaCl for 45 min). Fractions were collected and analyzed for activity with PNPA, isocarboxazid, and eprinomectin.

Results

***N*-Deacetylation of Eprinomectin by Selected Tissues.** One objective of this study was to determine which tissue, or tissues, was responsible for the *N*-deacetylation of eprinomectin. After incubation of eprinomectin in liver, kidney, spleen, small intestine, brain, muscle, lung and heart homogenates, and whole blood, the metabolic activity was found only in liver homogenate. In the previous study (Zeng et al., 1996), the enzyme activity was found mainly in liver microsomes, with very low activity in cytosol. At that time, we suspected the cytosol was contaminated with microsomes. In this study, we confirmed that cytosol did not show any *N*-deacetylation activities. This result led to a conclusion that the metabolism of eprinomectin was localized in liver microsomes.

Effect of pH on *N*-Deacetylation of Eprinomectin. Incubations of eprinomectin with rat liver microsomes were performed at a pH ranging from 4 to 10 with 0.5 pH unit intervals. The optimal pH was from 8 to 10. However, significant chemical hydrolysis of eprinomectin occurred at above pH 9. Thus, the experiment was routinely carried out at pH 8.

Kinetics of *N*-Deacetylation of Eprinomectin and Effect of NADPH. The rate of eprinomectin *N*-deacetylation was higher in female rats than in male rats (Table 1). This was also true in PB-treated rats. The apparent K_M values of these four different microsomes were similar. Because the rate of metabolism was higher in female rats, most metabolism studies were carried out with liver microsomes from female rats. The extent of *N*-deacetylation was the

TABLE 1

Kinetic parameters for *N*-deacetylation of eprinomectin by rat liver microsomes

Liver Microsomes	K_M	V_{max}
	μM	nmol/mg/min
Male	53 \pm 5	0.8 \pm 0.0
Female	70 \pm 16	5.0 \pm 0.6
Male treated with PB	90 \pm 16	2.8 \pm 0.3
Female treated with PB	102 \pm 25	5.7 \pm 0.8

Incubations were performed with 0.5 mg/ml microsomal protein for 30 min at an eprinomectin concentration ranging from 8 μM to 100 μM . Results are means \pm S.D.

same with and without NADPH (data not shown). Thus, the experiment was performed without NADPH.

Effect of Selective Inhibitors on *N*-Deacetylation of Eprinomectin. Table 2 shows the effect of different concentrations of various esterase inhibitors on the *N*-deacetylation of eprinomectin. Paraoxon and bis-4-nitrophenylphosphate (BNPP) strongly inhibited the hydrolysis activity at concentrations as low as 1 μM . The *N*-deacetylase activity was also inhibited by SKF525, but less effectively. Eserine, a cholinesterase inhibitor, did not inhibit the hydrolysis of eprinomectin at a concentration of 1×10^{-6} M, but nearly completely inhibited the activity at concentrations of 1×10^{-3} M and 1×10^{-4} M. NaF, an acetylcholinesterase inhibitor, slightly increased the activity. HgCl₂ decreased the activity to about 40% at both concentrations of 1×10^{-3} M and 1×10^{-4} M, but had no effect at lower concentrations. FeCl₃, CaCl₂, MgCl₂, and EDTA had little effect on the hydrolysis of eprinomectin.

***N*-Deacetylation of Eprinomectin by Microsomes from Rats Treated with Selective Inducers.** The *N*-deacetylation of eprinomectin was increased substantially by 300 to 600% in the liver microsomes from rats treated with pregnenolone-16 α -carbonitrile (PCN), dexamethasone (Dex), and PB; all are CYP3A inducers (Fig. 2). The hydrolysis activity was also increased by Aroclor 1254, isoniazid, clofibric acid, and perfluorodecanoic acid, but to a lesser extent. 3-Methylcholanthrene (3MC), streptozotocin, and β -naphthoflavone had little effect.

Correlation between Rate of *N*-Deacetylation of Eprinomectin and Other Known Esterase Substrates by Liver Microsomes from Untreated and Chemically Treated Rats. The rate of *N*-deacetylation of eprinomectin by microsomes from untreated and various chemically treated rats was compared with the rates of hydrolysis of PNPA, butanilcaine, palmitoyl-CoA, and isocarboxazid by the same microsomes. The hydrolysis of eprinomectin correlated well with that of isocarboxazid ($r = 0.92$, Fig. 3). On the other hand, no correlation was observed between *N*-deacetylation of eprinomectin and the metabolism of PNPA, butanilcaine, or palmitoyl-CoA (data not shown).

Chromatographic Separation of Esterases for Hydrolysis of PNPA, Isocarboxazid, and Eprinomectin. Two active enzyme fractions were resolved by gel filtration. Both fractions showed hydrolysis activity for PNPA, but only the low-molecular-weight fraction had activity for isocarboxazid and eprinomectin. From the ion exchange column with the low-molecular-weight fraction, two fractions eluted by 90 mM and 175 mM NaCl contained PNPA hydrolase activity, whereas only 175 mM NaCl eluate showed isocarboxazid and eprinomectin hydrolase activities (Fig. 4).

Discussion

Esterases are enzymes capable of catalyzing the hydrolysis of a wide range of esters, amides, and thioesters (Heymann, 1980; Satoh, 1987). Thus, they play important roles in the metabolism of various drugs and chemicals. Esterases have been identified in various mammalian tissues including blood, liver, kidney, small intestines, testis,

TABLE 2

Effect of various chemicals on hydrolysis rate of eprinomectin by rat liver microsomes

Chemicals	10 ⁻⁶ M	10 ⁻⁵ M	10 ⁻⁴ M	10 ⁻³ M
	% Remaining Activity			
Paraoxon	10	8	8	
BNPP	18	1	2	2
SKF525	78	33	7	3
Eserine	90	64	4	1.5
HgCl ₂	100	114	38	41
FeCl ₃		101	98	83
NaF		118	116	107
MgCl ₂		91	88	87
CaCl ₂		94	90	92
EDTA		94	95	86

Incubation solutions containing 10 μM of eprinomectin and 0.5 mg/ml microsomal protein were incubated for 45 min at 37°C. Microsomes were preincubated with inhibitors at 37°C for 10 min before the reaction was initiated by the addition of eprinomectin. Percentage of control is the percentage of eprinomectin metabolized in the presence of inhibitor relative to control samples that had no inhibitor. Each point represents an average of duplicates.

brains, central nervous system, and lung (Satoh, 1987). In our study, *N*-deacetylation of eprinomectin was detected only in liver microsomes.

Esterase activity including deacetylation could be mediated by cytochrome P-450 (Guengerich, 1987, 1988; Anderson et al., 1995). However, independence of NADPH in eprinomectin *N*-deacetylation by liver microsomes ruled out the involvement of cytochrome P-450. Aldridge (1953) classified esterases into three groups based on their interaction with organophosphates. "A" esterases hydrolyze organophosphates, "B" esterases are inhibited by them and include carboxylesterase and cholinesterase, and "C" esterases do not interact with organophosphates. Complete inhibition of eprinomectin *N*-deacetylation by paraoxon indicates that the enzyme system belongs to the B esterase class. The independence of Ca⁺⁺ and Mg⁺⁺ as cofactors, and insensitivity to EDTA and low concentrations of HgCl₂, suggest further their resemblance to a B esterase (Heymann, 1980; Walker and Mackness, 1983). Eserine, an inhibitor of cholinesterase (Simeon et al., 1988), inhibited the enzyme activity only at high concentrations, whereas NaF (Cimasoni, 1966), an inhibitor of acetylcholinesterase, increased the activity slightly. BNPP, which inhibits carboxylesterase at low concentrations but not cholinesterase (Simeon et al., 1988), strongly inhibited the hydrolysis of eprinomectin at concentrations as low as 10⁻⁶ M. Thus, a liver microsomal carboxylesterase is likely to be involved in the *N*-deacetylation of eprinomectin.

Multiple forms of carboxylesterase in rat liver microsomes have been purified and characterized by several groups (Mentlein et al., 1980; Robbi and Reaufay, 1983; Hosokawa et al., 1987; Morgan et al., 1994). Although each group has developed its own nomenclature for the isoforms, many enzymes purified by different groups seem to overlap with each other according to their enzymatic properties (Mentlein et al., 1987; Morgan et al., 1994). Hosokawa et al. (1987) isolated three isozymes of carboxylesterases from liver microsomes: RL1, RL2, and RH1. The amounts of RL1 and RH1 either are not different between sexes or are higher in male rats. Eprinomectin hydrolase activity, however, was higher in female rats. Furthermore, RL1 and RH1 were suppressed by Dex treatment (Hosokawa et al., 1993), whereas the hydrolysis of eprinomectin was increased markedly by treatment with Dex and PB (Fig. 2). Thus, RL1 and RH1 are not likely to be responsible for the hydrolysis of eprinomectin. RL2, on the other hand, was increased by both Dex and PB. In addition, the amount of RL2 also was greater in female rats than in male rats. Moreover, by comparing the hydrolysis activity by liver microsomes from rats treated with various P-450 inducers, eprinomectin did not correlate with palmitoyl-CoA, a specific substrate of RL1, or butanil-

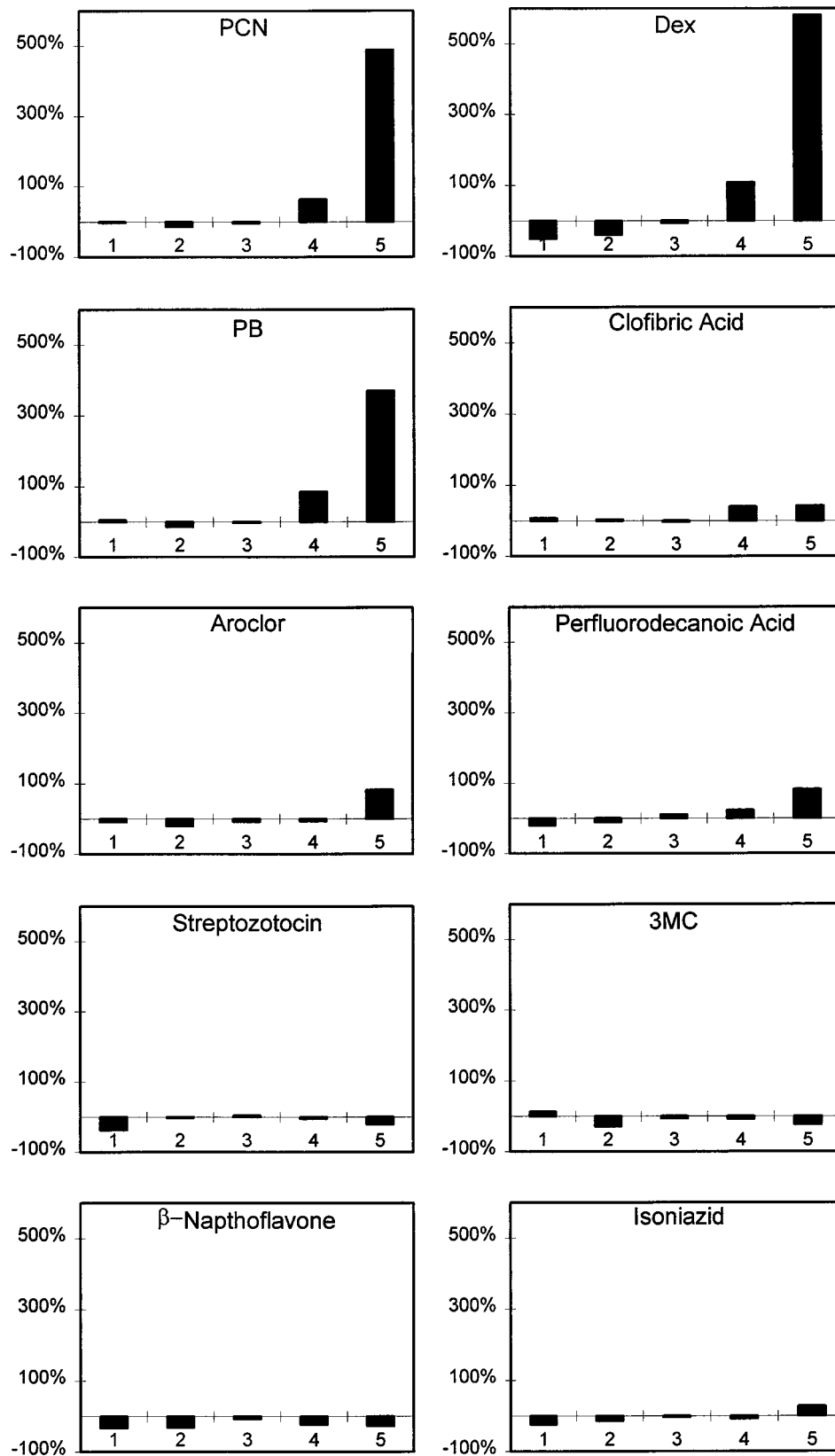


FIG. 2. Effect of induction on the rates of hydrolysis of eprinomectin and other esterase substrates by microsomes from rats treated with: 1, PNPA; 2, butanilicaine; 3, palmitoyl-CoA; 4, isocarboxazid; and 5, eprinomectin.

The data represent the percentage of hydrolysis activity in untreated male liver microsomes. Each bar represents an average of duplicates or triplicates except for palmitoyl-CoA, for which a single point was used.

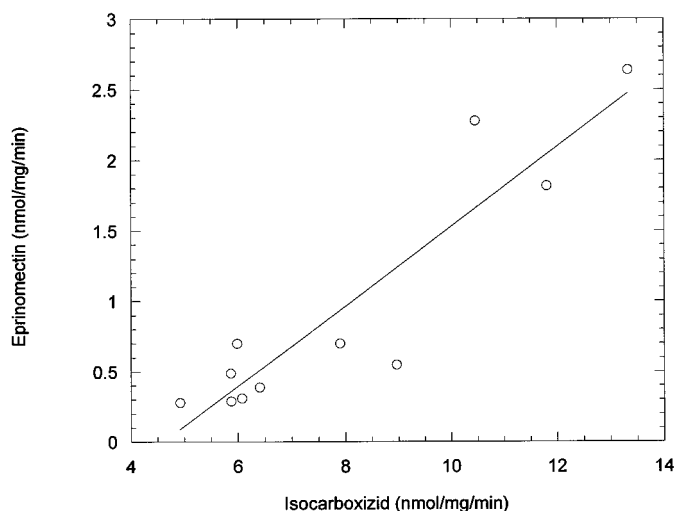


FIG. 3. Correlation of eprinomectin deacetylation and isocarboxazid hydrolysis by liver microsomes prepared from rats pretreated with different inducers.

Each point represents an average of duplicates for isocarboxazid and triplicates for eprinomectin. The line was drawn using linear regression analysis ($r = 0.92$).

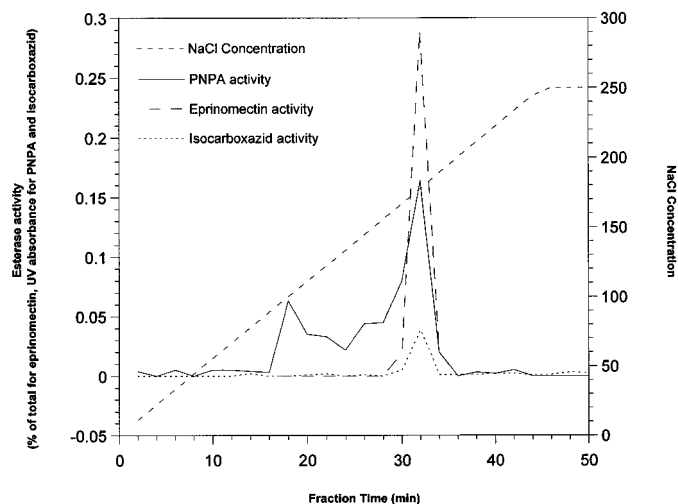


FIG. 4. Anion exchange chromatography of rat liver microsomal esterases for hydrolysis of PNPA, isocarboxazid, and eprinomectin.

Column fractions were assayed for esterase activities. The hydrolysis activity toward eprinomectin was calculated by the percentage of total radioactivity, whereas activities toward PNPA and isocarboxazid were based on UV absorbencies using a spectrophotometer.

icaine, a specific substrate of RH1, but correlated well with isocarboxazid, a substrate of RL2.

Esterases from liver microsomes were separated partially using a procedure similar to that of Hosokawa et al. (1987). Hosokawa et al. separated RH1 from RL1 and RL2 by gel filtration because RH1 was eluted as a trimer in the high-molecular-weight fraction. All these enzymes have hydrolysis activities toward PNPA. We also found that two fractions contained PNPA hydrolase activity, but only the low-molecular-weight fraction showed hydrolase activity toward eprinomectin. In their study, RL1 and RL2 were eluted by 30 mM and 150

mM NaCl from ion exchange chromatography, respectively. Similarly, we had two active fractions with PNPA hydrolase activities from ion exchange chromatography, and only the fraction with a higher concentration of NaCl showed isocarboxazid (RL2) and eprinomectin hydrolase activity. In other words, isocarboxazid hydrolase and eprinomectin hydrolase coeluted from both gel filtration and ion exchange chromatography. Furthermore, SDS-polyacrylamide gel electrophoresis analysis of the isolated active peak fraction (30–32 min, Fig. 4) showed a band with molecular weight of 66,000 (data not shown), which is similar to RL2 (~61,000). With the exception of a high-molecular-weight species, the 66-kDa protein is the predominant species observed. Thus, it appears that the enzyme system responsible for the hydrolysis of eprinomectin is either RL2 or very similar to RL2 purified by Hosokawa et al. To confirm this finding, further purification and characterization of the enzyme system are needed.

Acknowledgments. We express our sincere appreciation to Drs. Anthony Lu and Bruce Halley for invaluable comments, to Xiangli Yang for technical assistance, and to Dr. Allen Jones, Mrs. Yolanda Jakubowski, and Mr. Herbert Jenkins for supplying [^3H]AAB $_{1a}$.

References

- Aldridge WN (1953) Serum esterases (1) Two types of esterases (A and B) hydrolysing p-nitrophenyl acetate, propionate and butyrate, and a method for their determination. *Biochem J* **52**:110–117.
- Anderson CD, Wang J, Kumar GN, McMillan JM, Walle UK and Walle T (1995) Dexamethasone induction of Taxol metabolism in the rat. *Drug Metab Dispos* **23**:1286–1290.
- Berge RK (1979) Purification and characterization of a long-chain acyl-CoA hydrolase from rat liver microsomes. *Biochim Biophys Acta* **574**:321–333.
- Cimasoni G (1966) Inhibition of cholinesterases by fluoride *in vitro*. *Biochem J* **99**:133–137.
- Cvetovich RJ, Kelly DH, DiMichele LM, Shuman RF and Grabowski EJJ (1994) Syntheses of 4'-*epi*-amino-4"-deoxyavermectin B1. *J Org Chem* **59**:7704–7708.
- Guengerich FP (1987) Oxidative cleavage of carboxylic esters by cytochrome P-450. *J Biol Chem* **262**:8459–8462.
- Guengerich FP, Peterson LA and Böcker RH (1988) Cytochrome P-450-catalyzed hydroxylation and carboxylic acid ester cleavage of Hantzsch pyridine esters. *J Biol Chem* **263**:8176–8183.
- Heymann E (1980) Carboxylesterases and amides, in *Enzymatic Basis of Detoxification* (Jakoby WB ed) vol 11, pp 291–323, Academic Press, New York.
- Heymann E, Mentlein R and Rix H (1981) Hydrolysis of aromatic amides as assay for carboxylesterase-amidases. *Methods Enzymol* **77**:405–409.
- Hosokawa M, Hattori K and Satoh T (1993) Differential responses of rat hepatic microsomal carboxylesterase isozymes to glucocorticoids and pregnenolone 16 α -carbonitrile. *Biochem Pharmacol* **45**:2317–2322.
- Hosokawa M, Maki T and Satoh T (1987) Multiplicity and regulation of hepatic microsomal carboxylesterases in rats. *Mol Pharmacol* **31**:579–584.
- Krisch K (1966) Reaction of a microsomal esterase from hog-liver with diethyl p-nitrophenyl phosphate. *Biochim Biophys Acta* **122**:265–280.
- Mentlein R, Heiland S and Heymann E (1980) Simultaneous purification and comparative characterization of six serine hydrolases from rat liver microsomes. *Arch Biochem Biophys* **200**:547–559.
- Mentlein R, Ronai A, Robbi M, Heymann E and Deimling OV (1987) Genetic identification of rat carboxylesterases isolated in different laboratories. *Biochim Biophys Acta* **913**:27–38.
- Morgan, SEW, Yan B, Greenway DD, Petersen DR and Parkinson A (1994) Purification and characterization of two rat liver microsomal carboxylesterases (hydrolase A and B). *Arch Biochem Biophys* **315**:495–512.
- Robbi M and Reaufay H (1983) Purification and characterization of various esterases from rat liver. *Eur J Biochem* **137**:293–301.
- Satoh T (1987) Role of carboxylesterases in xenobiotic metabolism, in *Reviews in Biochemical Toxicology* (Hodgson E, Bend JR and Philpot RM eds) vol 8, pp 155–181, Elsevier, New York.
- Satoh T and Moroi K (1973) Comparison between procaine and isocarboxazid metabolism *in vitro* by a liver microsomal amidase-esterase. *Biochem Pharmacol* **24**:1517–1521.
- Shoop WL, Egerton JR, Eary CH, Haines HW, Michael BF, Mrozik H, Eskola P, Fisher MH, Slayton L, Ostlund DA, Skelly BJ, Fulton RK, Barth D, Costa S, Gregory LM, Campbell WC, Seward RL and Turner MJ (1996) Eprinomectin: A novel avermectin for use as a topical endectocide for cattle. *Int J Parasitol* **26**:1237–1242.
- Simeon V, Reiner E, Skrinjaric-Spoljar M and Drauthacker B (1988) Cholinesterases in rabbit serum. *Gen Pharmacol* **19**:849–853.
- Walker CH and Mackness MI (1983) Esterases: Problems of identification and classification. *Biochem Pharmacol* **32**:3265–3269.
- Zeng Z, Andrew NW, Green-Erwin ML and Halley BA (1996) Fate of 4'-*epi*-amino-4"-deoxyavermectin B1. *Drug Metab Dispos* **24**:572–578.