Biochemical Pharmacology, Vol. 42, Suppl., pp. S143–S150, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00

INDUCTION OF CYTOCHROME P450 ISOZYMES IN RAT LIVER BY METHYL *n*-ALKYL KETONES AND *n*-ALKYLBENZENES

EFFECTS OF HYDROPHOBICITY OF INDUCERS ON INDUCIBILITY OF CYTOCHROME P450

SUSUMU IMAOKA* and YOSHIHIKO FUNAE
Laboratory of Chemistry, Osaka City University Medical School, Osaka 545, Japan

(*Received* 24 *May* 1991; *accepted* 15 *August* 1991)

Abstract—The effects of methyl *n*-alkyl ketones and *n*-alkylbenzenes on hepatic cytochrome P450s *in vivo* and *in vitro* were investigated. Male rats were treated with acetone, methyl ethyl ketone, methyl *n*-propyl ketone, methyl *n*-butyl ketone, benzene, toluene, ethylbenzene, *n*-propylbenzene, or *n*-butylbenzene. The methyl *n*-alkyl ketones induced the metabolic activities of hepatic microsomes toward aminopyrine, 7-ethoxycoumarin, and aniline. *n*-Alkylbenzenes induced aminopyrine and 7-ethoxycoumarin metabolic activities. Testosterone 2β- and 6β-hydroxylation activities were induced by ketones with a long side chain such as methyl *n*-butyl ketone. Testosterone 2α-hydroxylation activity was decreased by treatment with methyl *n*-butyl ketone. Testosterone 16β-hydroxylation activity was induced by treatment with methyl *n*-alkyl ketones. The inducibility was dependent on the length of the side chain. Testosterone 16β-hydroxylation activity also was induced by *n*-alkylbenzenes. These results indicate that the levels of multiple forms of cytochrome P450 were changed by treatment with these chemicals. P450IIE1, an acetone-inducible form, was induced by methyl *n*-alkyl ketones or *n*-alkylbenzenes. The inducibility did not depend on the length of the side chain of these chemicals. P450IIB1 and IIB2, both phenobarbital-inducible forms, were induced with methyl *n*-alkyl ketones and *n*-alkylbenzenes to an extent depending on the length of the side chain of these chemicals. Thus, the hydrophobicity of the inducer affected phenobarbital-type induction but not the induction of P450IIE1. We further investigated the interactions of ketone and benzene derivatives with cytochrome P450 *in vitro*. Testosterone hydroxylation activities of hepatic microsomes were measured in the presence of methyl *n*-alkyl ketones and *n*-alkylbenzenes. Methyl *n*-alkyl ketones inhibited testosterone 16β-hydroxylation activity. *n*-Alkylbenzenes inhibited 2β-, 6β-, 15α-, 16α-, and 16β-hydroxylation activities. Testosterone hydroxylation activities were inhibited by these chemicals depending on the length of the side chain. *n*-Alkylbenzenes were stronger inhibitors than methyl *n*-alkyl ketones, *n*-Butylbenzene was the strongest inhibitor of these activities. These results indicate that hydrophobicity was important in the interaction of these chemicals with cytochrome P450, and that there is some relationship between the inducibility of cytochrome P450 and its interaction with inducers.

Administration of xenobiotics such as phenobarbital and 3-methylcholanthrene to animals induces individual cytochrome P450s in hepatic microsomes. The induction of cytochrome P450 is a regulatory phenomenon of clinical and toxicological importance [1, 2]. Phenobarbital and 3-methylcholanthrene are the inducers that have been studied the most [1, 2]. The mechanism of induction by 3-methylcholanthrene and similar compounds involves their stereospecific binding to a cytosol protein and the nuclear uptake of inducer–protein complexes, that stimulate gene expression [3, 4]. Such inducers are structurally related to each other. On the contrary, a large variety of compounds, without structural relatedness cause phenobarbital-like induction [5]. The lack of a discernible structure–activity relationship suggested that the induction mechanism of phenobarbital is different from that of 3-methylcholanthrene. The molecular mechanism by which phenobarbital induces cytochrome P450 is not known.

Very simple molecules (ethanol and acetone) induce some cytochrome P450s, especially P450IIE1 [6–8]. Structurally diverse compounds such as pyrazole, imidazole, and benzene also induce this cytochrome P450 [9–11]. As in phenobarbital-type induction, structural requirements for the inducers of P450IIE1 have not been identified. Sinclair *et al.* [12] report that the induction of cytochrome P450 in culture hepatocytes by alcohols and 4-substituted pyrazoles depends on their hydrophobicity; the inducibility of cytochrome P450 increases as the hydrophobicity of these chemicals increases. However, which of the many isozymes of cytochrome P450s are induced by these chemicals and how the hydrophobicity of these chemicals affects the levels of the cytochrome P450s have not been clarified. In the present study we investigated the changes caused by methyl *n*-alkyl ketones and *n*-alkylbenzenes in the levels of eleven different forms of cytochrome P450 in rat hepatic microsomes.

4-*n*-Alkyl-methylenedioxybenzenes have been studied extensively as inhibitors and inducers of

* Corresponding author: Dr. Susumu Imaoka, Laboratory of Chemistry, Osaka City University Medical School, 1-4-54 Asahimachi, Abeno-ku, Osaka 545, Japan. Tel. 06-645-2081 (direct); FAX 06-645-2081.

cytochrome P450 [13, 14]. Treatment of animals with these chemicals results in the formation of metabolite–cytochrome P450 complexes and then in the induction of cytochrome P450 [13]. These chemicals induce some forms of cytochrome P450; which form of cytochrome P450 is induced depends on the length of the alkyl chain [14]. Inducibility seems to be related to the ability of these chemicals to form a complex with cytochrome P450 [13]. Many ligands and inhibitors of cytochrome P450 are good inducers [15]. The inhibition of certain cytochrome P450s *in vivo* may induce a specific cytochrome P450. Therefore, we also investigated the inhibition by ketone and benzene derivatives in testosterone hydroxylation of hepatic microsomes to clarify the relationship between the inducibility of cytochrome P450 by ketone and benzene derivatives and the binding capacity of these chemicals to cytochrome P450.

MATERIALS AND METHODS

Chemicals. Testosterone was obtained from the Sigma Chemical Co. (St. Louis, MO). NADPH was obtained from the Oriental Yeast Co. (Tokyo, Japan). Acetone, methyl ethyl ketone (MEK*), methyl *n*-propyl ketone (MPK), methyl *n*-butyl ketone (MBK), benzene, toluene, ethylbenzene, *n*-propylbenzene (PrB), and *n*-butylbenzene (BuB) were obtained from the Kasei Kogyo Co. (Tokyo, Japan). Other reagents and organic solvents were obtained from Wako Pure Chemical Industries (Tokyo, Japan).

Treatment of animals. Male Sprague–Dawley rats weighing 230–250 g were obtained from Charles River (Tokyo, Japan). Acetone, MEK, MPK, MBK, benzene, toluene, ethylbenzene, PrB, and BuB (5 mmol/kg, dissolved in corn oil) were given intraperitoneally daily for 4 days. The dose of 5 mmol/kg was chosen because a dose of more than 5 mmol/kg MBK killed the rats. Control rats were given corn oil only. Hepatic microsomes were prepared as reported elsewhere [16].

Assay of the levels of cytochrome P450 isozymes. Antibody against purified cytochrome P450 was raised as described previously [17] in a female Japanese White rabbit obtained from Biotech (Saga, Japan). The characterization of antibodies against P450IIA1, IIA2, IIB1, IIB2, IIC6, IIC11, IIC13, IIE1, IIIA2, and IVA3 was reported elsewhere [18]. P450IIC7 (our name, P450 UT-1b) was newly purified by the same method [19] as that used in the purification of P450IIIA2 (our name, P450 PB-1). Briefly, hepatic microsomes of untreated male rats were fractionated with polyethylene glycol, chromatographed on an octylamino-Sepharose column, and put on HPLC with a DEAE-5PW column (Tosoh, Tokyo, Japan). A pass-through fraction from a DEAE-5PW column was put on HPLC with an ES-502CP column (CM-type, Asahi Chemicals, Tokyo, Japan), and the P450 UT-1b fraction was further purified by a KB-column (hydroxyapatite column, Koken, Tokyo, Japan). Anti-P450 UT-1b antibody was prepared as were the other antibodies described above [17]. Anti-P450 UT-1b antibody reacted with P450IIC6, IIC11, and IIC13 as well as with P450 UT-1b (P450IIC7). Therefore, this antibody was purified by affinity chromatography with a Sepharose to which cytochrome P450 was bound as described previously [18]. Measurement of cytochrome P450 by immunoblotting was reported elsewhere [17, 18].

Metabolic activity. The activities of aminopyrine N-demethylation, 7-ethoxycoumarin O-dealkylation, aniline hydroxylation, and testosterone hydroxylation were measured as described previously [16–19].

RESULTS

Changes in catalytic activity of hepatic microsomes of rats treated with methyl n-alkyl ketones. Male rats were treated with acetone, MEK, MPK, or MBK. The content of total cytochrome P450 and the catalytic activities of hepatic microsomes are shown in Table 1. The content of total cytochrome P450 measured photometrically did not change much with treatment. However, the activities of aminopyrine N-demethylation, aniline hydroxylation, and 7-ethoxycoumarin O-dealkylation were increased by treatment with methyl *n*-alkyl ketones. The increase in 7-ethoxycoumarin O-dealkylation activity was dependent on side-chain length but those in aminopyrine and aniline oxidation activities were not. Changes in the testosterone hydroxylation activities of hepatic microsomes caused by the treatment with methyl *n*-alkyl ketones are shown in Table 1. Testosterone, 7α-, 15α-, and 16α-hydroxylation activities did not change significantly. Testosterone 2β-, 6β-, and 16β-hydroxylation activities were induced. Ketones having a long side chain were effective for induction of testosterone 2β- and 6β-hydroxylation activities. Testosterone 16β-hydroxylation activity increased depending on the increase in the length of the side chain in methyl *n*-alkyl ketones. Testosterone 2α-hydroxylation activity was decreased by treatment with MBK. These results show that treatment with ketones changed the population of cytochrome P450 isozymes in hepatic microsomes.

Changes in the levels of cytochrome P450 in hepatic microsomes caused by treatment with methyl n-alkyl ketones. Eleven forms of cytochrome P450s were assayed with immunoblotting (Table 1). The level of P450IIC11, a typical male-specific form [21], was decreased by treatment with MBK, in parallel with a decrease in testosterone 2α-hydroxylation activity, which is catalyzed by P450IIC11 [22]. P450IIA2 and IIC13 are also male-specific forms [23, 24]. The levels of these forms were not changed much by treatment with ketones. P450IIC7 is present in the hepatic microsomes of rats of both sexes. It was not induced by ketones. P450IIC6 and IIIA2 are major constitutive forms in male rats and are phenobarbital-inducible [25]. These forms were induced by the ketones. P450IIIA2 is a constitutive testosterone 6β-hydroxylase [19]. The increase in the level of this

* Abbreviations: MEK, methyl ethyl ketone; MPK, methyl *n*-propyl ketone; MBK, methyl *n*-butyl ketone; PrB, *n*-propylbenzene; and BuB, *n*-butylbenzene.

Table 1. Changes in the catalytic activities and in the levels of cytochrome P450s caused by treatment with methyl *n*-alkyl ketones

Treatment	Control	Acetone	MEK	MPK	MBK
Total P450	0.65 ± 0.05	0.67 ± 0.11	0.67 ± 0.08	0.74 ± 0.10	0.70 ± 0.05
Aminopyrine	2.40 ± 0.50	4.37 ± 0.91*	4.14 ± 0.70*	4.46 ± 0.92*	4.37 ± 0.82*
Aniline	0.283 ± 0.044	0.382 ± 0.032*	0.347 ± 0.036†	0.433 ± 0.063*	0.421 ± 0.070*
7-Ethoxycoumarin	3.62 ± 0.54	3.76 ± 0.13	4.18 ± 0.53	5.78 ± 0.88*	6.01 ± 1.24*
Testosterone					
2α	0.684 ± 0.114	0.671 ± 0.107	0.585 ± 0.114	0.624 ± 0.238	0.431 ± 0.158†
2β	0.140 ± 0.039	0.151 ± 0.029	0.162 ± 0.033	0.221 ± 0.062†	0.240 ± 0.056†
6β	0.952 ± 0.197	0.959 ± 0.176	1.052 ± 0.232	1.508 ± 0.363†	1.445 ± 0.341†
7α	0.056 ± 0.006	0.065 ± 0.012	0.064 ± 0.021	0.053 ± 0.010	0.062 ± 0.013
15α	0.040 ± 0.007	0.046 ± 0.010	0.040 ± 0.013	0.048 ± 0.016	0.056 ± 0.017
16α	1.090 ± 0.203	1.070 ± 0.225	1.092 ± 0.130	1.166 ± 0.364	1.067 ± 0.347
16β	0.058 ± 0.006	0.057 ± 0.008	0.095 ± 0.023	0.180 ± 0.041	0.250 ± 0.106†
P450 form					
IIA1	7.0 ± 1.3	9.7 ± 2.2†	8.8 ± 2.3	8.3 ± 1.9	7.9 ± 1.5
IIA2	10.4 ± 2.3	12.3 ± 3.5	9.3 ± 2.4	10.0 ± 2.4	11.7 ± 2.8
IIB1	<0.5	0.9 ± 0.4	5.7 ± 2.7	28.7 ± 5.2	44.3 ± 9.4
IIB2	3.8 ± 1.2	6.0 ± 1.5	10.7 ± 1.8	21.5 ± 3.6	29.3 ± 6.2
IIC6	52.1 ± 17.7	67.9 ± 7.8	87.3 ± 17.8†	85.3 ± 16.5†	93.4 ± 16.9*
IIC7	21.9 ± 3.3	26.2 ± 4.3	28.0 ± 7.1	22.3 ± 5.3	24.8 ± 5.8
IIC11	457.0 ± 52.6	469.6 ± 44.2	459.0 ± 36.3	411.8 ± 29.7	343.8 ± 46.3†
IIC13	171.4 ± 35.8	171.7 ± 39.9	149.6 ± 18.0	158.4 ± 19.5	159.7 ± 24.5
IIE1	49.8 ± 9.6	157.7 ± 28.2*	92.7 ± 15.0*	105.6 ± 19.6*	102.6 ± 14.8*
IIIA2	87.9 ± 13.8	103.0 ± 20.0	107.0 ± 17.2	151.1 ± 23.3*	141.4 ± 28.7*
IVA3	17.6 ± 3.2	18.2 ± 2.9	13.8 ± 3.0	19.6 ± 3.0	16.7 ± 2.8

The content of total cytochrome P450 was measured photometrically and is expressed as nanomoles per milligram of protein. Catalytic activities are expressed as nanomoles of product per minute per milligram of protein. The levels of individual cytochrome P450s were measured by immunoblotting. The values are expressed as picomoles of cytochrome P450 per milligram of protein. Assays were done on five or six different preparations of microsomes. Values are expressed as means ± SD. 2α, 2β, 6β, 7α, 15α, 16α, and 16β indicate the hydroxylation sites of testosterone by hepatic microsomes. The designations given to the rat hepatic cytochrome P450s described in this study can be related to the standardized gene designations by Nebert *et al.* [20]. Abbreviations: MEK, methyl ethyl ketone; MPK, methyl *n*-propyl ketone; and MBK, methyl *n*-butyl ketone.

* Significantly different from control or group indicated in the table, $P < 0.01$.

† Significantly different from control or group indicated in the table, $P < 0.05$.

form explained the increase in testosterone 6β-hydroxylation activity in the hepatic microsomes caused by the treatment with ketones. P450IIB1 and IIB2 are typical phenobarbital-inducible forms. The level of P450IIB1 in the hepatic microsomes of control rats was very low and P450IIB2 was detected at a slightly higher level. Both forms were strongly induced by ketones. These results reflected the increases in testosterone 16β-hydroxylation and aminopyrine N-demethylation activities of hepatic microsomes caused by ketones [25], and suggested that methyl *n*-alkyl ketone was a phenobarbital-type inducer. The relationship of inducibility of P450IIB1 and IIB2 to the hydrophobicity of methyl *n*-alkyl ketones is shown in Fig. 1. Inducibility of these forms became higher with the increase in hydrophobicity (partition coefficient between octanol and water) of ketone derivatives. Acetone induces P450IIE1. Other ketones also induced P450IIE1. Unlike P450IIB1 and IIB2, the inducibility of P450IIE1 by ketones was not dependent on the length of the side chain or hydrophobicity (Fig. 1). Diabetes as well as acetone induces P450IIE1 [27]. Diabetes also strongly induces P450IVA3 [28], which was purified from the hepatic microsomes of diabetic rats and has high lauric acid hydroxylation activity [28, 29]. The level of P450IVA3 was not changed by ketones. P450IIA1 (testosterone 7α-hydroxylase [30]), which is abundant in hepatic microsomes of immature rats, was not induced much by ketones. P450IA1 and IA2, typical 3-methylcholanthrene-inducible forms, were not induced by ketones (data not shown).

Changes in catalytic activity of hepctic microsomes of rats treated with n-*alkylbenzenes*. The content of total cytochrome P450 measured photometrically was not changed by treatment with *n*-alkylbenzenes (Table 2). The aminopyrine N-demethylation and 7-ethoxycoumarin O-dealkylation activities of hepatic microsomes were induced with *n*-alkylbenzenes but *n*-alkylbenzenes did not increase aniline hydroxylation activity much (Table 2). Testosterone 2β- and 6β-hydroxylation activities were both induced by PrB and by BuB with a long side chain (Table 2). Unlike induction by ketones, *n*-alkylbenzenes induced testosterone 7α-hydroxylation activity.

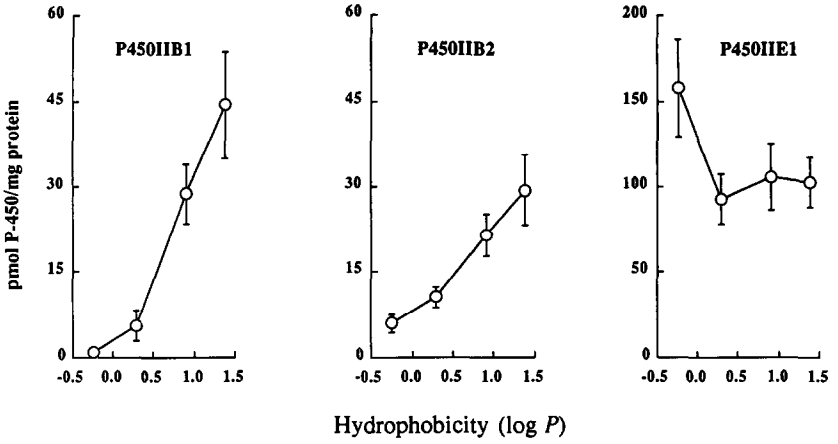

Fig. 1. Relationship of cytochrome P450 levels induced by methyl *n*-alkyl ketones to hydrophobic constant. *P* is a hydrophobic parameter measured as the partition coefficient between octanol and water [26]. Log *P* values for acetone, methyl ethyl ketone, methyl *n*-propyl ketone, and methyl *n*-butyl ketone are −0.24, 0.29, 0.91, and 1.38, respectively. The levels of cytochrome P450 are expressed as means ± SD of five or six different preparations of microsomes.

Testosterone 16β-hydroxylation activity was strongly induced by *n*-alkylbenzenes. Testosterone 2α-, 15α-, and 16α-hydroxylation activities were not increased by treatment with *n*-alkylbenzenes.

Changes in the levels of cytochrome P450 by treatment with n-alkylbenzenes. The levels of cytochrome P450s in hepatic microsomes of rats treated with *n*-alkylbenzenes are shown in Table 2. Except for BuB, the *n*-alkylbenzenes decreased the level of P450IIC11 but did not decrease the level of P450IIA2 or IIC13. These forms are constitutive male-specific forms [23, 24]. P450IIIA2 was induced depending on the length of the side chain of the *n*-alkylbenzene, reflecting the increase of testosterone 2β- and 6β-hydroxylation activities of hepatic microsomes [19]. P450IIC6 was also induced by the *n*-alkylbenzenes other than benzene. Inducibility did not depend on the length of side chain of the *n*-alkylbenzene. As with methyl *n*-alkyl ketone, P450IIB1 and IIB2 were strongly induced by the *n*-alkylbenzenes. Inducibility of these forms became higher with the increase in hydrophobicity of *n*-alkylbenzene (Fig. 2). However, the inducibility of testosterone 16β-hydroxylation activity catalyzed by P450IIB1 did not depend on the increase in the length of the side chain in the *n*-alkylbenzene. The reason for this discrepancy is not known. Some metabolites of *n*-alkylbenzene may bind to cytochrome P450IIB1 and inhibit the testosterone 16β-hydroxylation activity [9]. Like the ketones, *n*-alkylbenzenes induced P450IIE1 but did not induce P450IIA1 and IVA3 much. P450IA1 and IA2 were not induced by *n*-alkylbenzenes (data not shown).

Inhibition of testosterone hydroxylation activity in hepatic microsomes by methyl n-alkyl ketones and n-alkylbenzenes. Usually inhibitors or ligands of cytochrome P450 such as metyrapone are good inducers of cytochrome P450. As described above, methyl *n*-alkyl ketones and *n*-alkylbenzenes were phenobarbital-type inducers and the inducibility was changed by the length of the side chain of these ketone and benzene derivatives. So, to investigate the relationship between the inducibility of cytochrome P450 and the interaction of these chemicals with cytochrome P450, the testosterone hydroxylation activities of hepatic microsomes of rats treated with phenobarbital were measured in the presence of a methyl *n*-alkyl ketone or *n*-alkylbenzene (Fig. 3). The testosterone 16β-hydroxylation activity of hepatic microsomes was inhibited efficiently by either kind of compound. *n*-Alkylbenzenes with a long side chain were especially effective inhibitors. The strength of inhibition depended on the length of the side chain in the ketone and benzene derivatives. Testosterone 16β-hydroxylation is catalyzed by P450IIB1. The inducibility of this form by benzene and ketone derivatives seems to correlate with inhibitory effect. Testosterone 6β-hydroxylation activity (catalyzed by P450IIIA2) is also induced by phenobarbital. This activity was inhibited effectively by *n*-alkylbenzenes. Testosterone 7α-hydroxylation activity (catalyzed by P450IIA1 [24]) and testosterone 2α-hydroxylation activity (catalyzed by P450IIC11) were inhibited weakly by ketone and benzene derivatives. However, testosterone 15α-hydroxylation activity (catalyzed by P450IIA2 [24]) was inhibited strongly by *n*-alkylbenzene. The strength of the inhibition of testosterone hydroxylation activities by methyl *n*-alkyl ketones and *n*-alkylbenzenes was correlated with the length of the side chain in methyl *n*-alkyl ketones and *n*-alkylbenzenes (hydrophobicity of these chemicals).

DISCUSSION

Acetone is a typical inducer of P450IIE1. In this study, acetone induced P450IIE1 more efficiently than the other inducers used. Other ketones and the

Table 2. Changes in the catalytic activities and in the levels of cytochrome P450s caused by treatment with *n*-alkylbenzenes

Treatment	Control	Benzene	Toluene	EtB	PrB	BuB
Total P450	0.65 ± 0.05	0.59 ± 0.07	0.62 ± 0.09	0.67 ± 0.06	0.53 ± 0.05	0.61 ± 0.06
Aminopyrine	2.40 ± 0.50	2.82 ± 0.54	3.89 ± 0.45*	4.28 ± 0.28*	3.02 ± 0.43	4.43 ± 0.71*
Aniline	0.283 ± 0.044	0.212 ± 0.058	0.278 ± 0.042	0.342 ± 0.044†	0.308 ± 0.041	0.297 ± 0.047
7-Ethoxycoumarin	3.62 ± 0.54	3.12 ± 0.59	4.32 ± 1.02	5.44 ± 1.11†	5.44 ± 0.53*	5.72 ± 0.47*
Testosterone						
2α	0.684 ± 0.114	0.393 ± 0.117*	0.564 ± 0.084	0.556 ± 0.134	0.390 ± 0.097*	0.555 ± 0.079
2β	0.140 ± 0.039	0.117 ± 0.027	0.180 ± 0.068	0.166 ± 0.094	0.212 ± 0.025*	0.205 ± 0.022*
6β	0.952 ± 0.197	0.701 ± 0.172	1.126 ± 0.340	1.240 ± 0.288	1.288 ± 0.160†	1.353 ± 0.146†
7α	0.056 ± 0.006	0.032 ± 0.010*	0.048 ± 0.010	0.080 ± 0.012*	0.071 ± 0.014	0.084 ± 0.021†
15α	0.040 ± 0.007	0.034 ± 0.005	0.045 ± 0.009	0.052 ± 0.005	0.040 ± 0.002	0.051 ± 0.009
16α	1.090 ± 0.203	0.643 ± 0.183*	0.998 ± 0.149	1.005 ± 0.259	0.828 ± 0.177	1.057 ± 0.102
16β	0.058 ± 0.006	0.059 ± 0.011	0.113 ± 0.033*	0.275 ± 0.047*	0.220 ± 0.034*	0.174 ± 0.047*
P450 form						
IIA1	7.0 ± 1.3	5.2 ± 1.2	5.2 ± 1.0	7.0 ± 1.7	7.7 ± 2.0	9.8 ± 1.5†
IIA2	10.4 ± 2.3	11.9 ± 2.3	10.1 ± 2.7	8.2 ± 2.8	7.4 ± 2.4	11.1 ± 3.2
IIB1	<0.5	3.4 ± 2.7	10.4 ± 2.3	30.9 ± 9.4	38.4 ± 6.0	52.8 ± 12.2
IIB2	3.8 ± 1.2	5.3 ± 1.8	10.9 ± 2.0	25.1 ± 7.2	30.3 ± 6.5	35.6 ± 7.4
IIC6	52.1 ± 17.7	54.8 ± 9.3	82.9 ± 10.3*	81.9 ± 11.3†	76.9 ± 12.8†	80.0 ± 13.0†
IIC7	21.9 ± 3.3	19.2 ± 4.0	17.5 ± 5.0	16.8 ± 5.3	19.6 ± 5.6	17.3 ± 2.5
IIC11	457.0 ± 52.6	261.2 ± 36.7*	345.2 ± 38.5*	329.4 ± 33.0*	357.0 ± 28.8*	397.2 ± 38.5
IIC13	171.4 ± 35.8	197.0 ± 32.2	234.1 ± 37.6†	190.3 ± 32.5	151.6 ± 22.5	142.9 ± 25.3
IIE1	49.8 ± 9.6	69.0 ± 18.2	74.7 ± 15.2†	78.3 ± 17.6†	80.4 ± 12.8*	87.9 ± 16.2*
IIIA2	87.9 ± 13.8	80.9 ± 9.3	94.6 ± 18.6	110.0 ± 15.2†	120.3 ± 16.9†	139.8 ± 20.1*
IVA3	17.6 ± 3.2	15.8 ± 4.4	18.0 ± 1.6	17.9 ± 3.0	19.7 ± 2.6	18.4 ± 2.7

The content of total cytochrome P450 was measured photometrically and is expressed as nanomoles per milligram of protein. Catalytic activities are expressed as nanomoles of product per minute per milligram of protein. The levels of individual cytochrome P450s were measured by immunoblotting. The values are expressed as picomoles of cytochrome P450 per milligram of protein. Assays were done of five or six different preparations of microsomes. Values are expressed as means ± SD. 2α, 2β, 6β, 7α, 15α, 16α, and 16β indicate the hydroxylation sites of testosterone by hepatic microsomes. The designations given to the rat hepatic cytochrome P450s described in this study can be related to the standardized gene designations by Nebert *et al.* [20]. Abbreviations: EtB, ethylbenzene; PrB, *n*-propylbenzene; and BuB, *n*-butylbenzene.

* Significantly different from control or group indicated in the table, $P < 0.01$.

† Significantly different from control or group indicated in the table, $P < 0.05$.

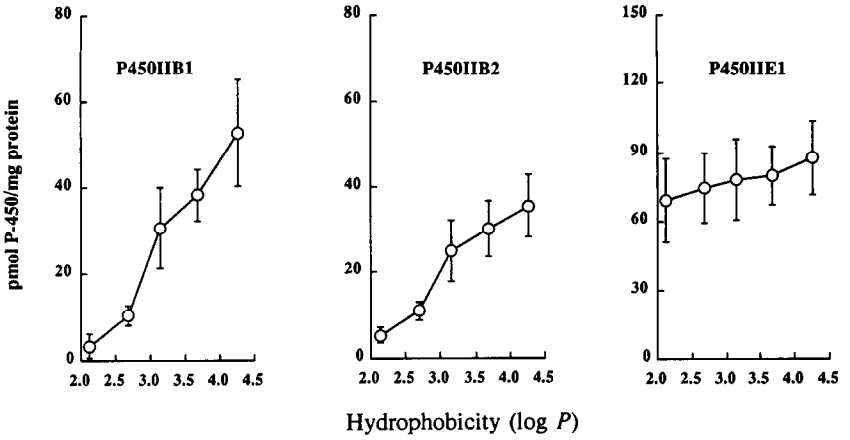

Fig. 2. Relationship of cytochrome P450 levels induced by *n*-alkylbenzenes to hydrophobic constant. *P* is a hydrophobic parameter measured as the partition coefficient between octanol and water [26]. Log *P* values for benzene, toluene, ethylbenzene, *n*-propylbenzene, and *n*-butylbenzene are 2.13, 2.69, 3.15, 3.68, and 4.26, respectively. The levels of cytochrome P450 are expressed as means ± SD of five or six different preparations of microsomes.

n-alkylbenzenes also induced P450IIE1, although the inducibility with benzene with the dose used in this study was low, and the level of P450IIE1 in rats treated with benzene was not significantly different from that in control rats. The inducibility of P450IIE1 was not dependent on the length of the side chain (hydrophobicity) in the ketone and benzene derivatives (Figs. 1 and 2). These chemicals also strongly induced P450IIB1 and IIB2, and the inducibility was dependent on the hydrophobicity of the inducers (Figs. 1 and 2). As Sinclair *et al.* [12] noted, hydrophobicity is necessary for induction of these cytochrome P450s. Song *et al.* [31] indicated that induction of P450IIE1 by acetone involves activation of post-transcriptional levels, since induction of the protein assayed immunochemically was not correlated to a proportional increase in the amount of the corresponding mRNA [31]. On the other hand, acetone treatment of rats increases the levels of P450IIB1 and IIB2 proteins and also the level of the corresponding mRNA [8]. Our results and these reported findings indicate that the induction mechanism of P450IIE1 by ketone and benzene derivatives is different from that of P450IIB1 (IIB2). P450IIE1 is induced by diabetes and starvation as well as by treatment with these chemicals [27]. The induction mechanism of P450IIE1 by acetone is different from that by diabetes and starvation, which increase the amount of the corresponding mRNA of P450IIE1 [8]. Diabetes and starvation induce P450IVA3 (lauric acid hydroxylase) [28, 29]. However, ketone and benzene derivatives did not induce P450IVA3. The induction mechanisms of P450IIE1 and IVA3 are different.

Murray *et al.* [13] suggested that the formation of strong substrate–cytochrome P450 complexes induces the complexed cytochrome P450 isozyme by providing stabilization against degradation or by increasing the capacity of cytochrome P450 to compete for a limiting pool of free heme. Fonné and Meyer [32] suggested that substrate-binding to a specific cytochrome P450 could increase the concentration of endogenous substrates that would later directly or indirectly mediate the induction response. P450IIB1 and IIB2 may be induced by ketone and benzene derivatives by these mechanisms. In fact, ketone and benzene derivatives that had long side chains efficiently induced P450IIB1 and IIB2 and also strongly inhibited testosterone 16β-hydroxylation activity, which is catalyzed by P450IIB1 (IIB2) in hepatic microsomes. *n*-Alkylbenzenes were more efficient inhibitors than methyl *n*-alkyl ketones and the *n*-alkylbenzenes were also more efficient inducers of P450IIB1 and IIB2 than the latter. Strong hydrophobicity is necessary for induction and inhibition of these forms.

In this study, we observed a discrepancy in the testosterone 16β-hydroxylation activity and the level of P450IIB1 in hepatic microsomes of rats treated with *n*-alkylbenzenes but did not observe such a discrepancy with ketone derivatives. The levels of P450IIB1 correlated with the testosterone 16β-hydroxylation activity in rats treated with ketone derivatives. The binding capacity of benzene derivatives to cytochrome P450 became higher with the increase in their hydrophobicity to judge from results of an inhibition study (Fig. 3). The discrepancy between testosterone 16β-hydroxylation activity and the level of P450IIB1 was large in benzene derivatives with a long side chain. Murray *et al.* [13] noted that treatment of rats with alkoxymethylenedioxybenzenes, which have long side chains, produces the metabolite–cytochrome P450 complex [13]. Benzene is metabolized by cytochrome P450 to produce active radical species and binds to proteins [9]. These results suggested that a benzene metabolite and cytochrome P450 form complexes.

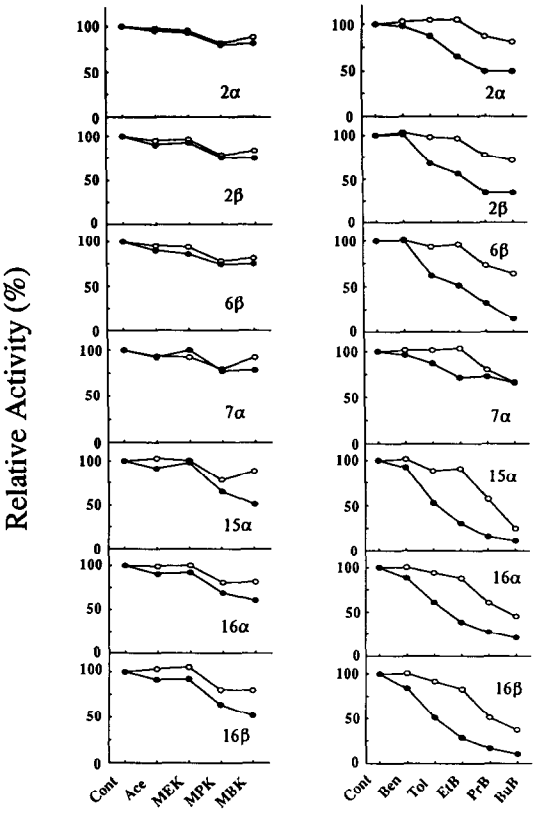

Fig. 3. Effects of methyl *n*-alkyl ketones and *n*-alkylbenzenes on the testosterone hydroxylation activity of hepatic microsomes. Methyl *n*-alkyl ketones and *n*-alkylbenzenes were added to a testosterone metabolic system constituted with hepatic microsomes (100 μg) of male rats treated with phenobarbital, testosterone (0.5 mM), and NADPH (0.2 mM). Open and closed circles indicate the addition of 0.25 and 2.5 mM ketone and benzene derivatives, respectively. 2α, 2β, 6β, 7α, 15α, 16α, and 16β indicate the hydroxylation sites of testosterone by hepatic microsomes. Abbreviations: Cont, control; Ace, acetone, MEK, methyl ethyl ketone; MPK, methyl *n*-propyl ketone, MBK, methyl *n*-butyl ketone; Ben, benzene; Tol, toluene, EtB, ethylbenzene; PrB, *n*-propylbenzene; and BuB, *n*-butylbenzene.

Details of the mechanisms which would account for the different effects of methyl *n*-alkyl ketones and *n*-alkylbenzenes on P450IIB1, IIB2, and IIE1 remain to be elucidated, but it is clear that hydrophobicity is important for the induction of P450IIB1 and IIB2 and that the induction mechanisms of P450IIB1 (IIB2) and IIE1 by these chemicals are different.

Acknowledgements—We thank Dr. T. Nasu of Shinshu University for her helpful discussion and Misses M. Ohki, K. Matsuyama and M. Minami for technical assistance.

REFERENCES

1. Oritz de Montellano PR (Ed.), *Cytochrome P-450: Structure, Mechanism, and Biochemistry*. Plenum, New York, 1986.
2. Miners J, Birkett DJ, Drew R and McManus M (Eds.), *Microsomes and Drug Oxidations*. Taylor & Francis, New York, 1987.
3. Poland AP, Glover E, Robinson JR and Nebert DW, Genetic expression of aryl hydrocarbon hydroxylase activity. *J Biol Chem* **249**: 5599–5606, 1974.
4. Nebert DW, Eisen HJ, Negishi M, Lang MA, Hjelmeland LM and Okey AB, Genetic mechanisms controlling the induction of polysubstrate monooxygenase (P-450) activities. *Annu Rev Pharmacol Toxicol* **21**: 431–462, 1981.
5. Conney AH. Pharmacological implications of microsomal enzyme induction. *Pharmacol Rev* **19**: 317–366, 1967.
6. Ryan DE, Koop DR, Thomas PE, Coon MJ and Levin W, Evidence that isoniazid and ethanol induce the same microsomal cytochrome P-450 in rat liver, an isozyme homologous to rabbit liver cytochrome P-450 isozyme 3a. *Arch Biochem Biophys* **246**: 633–644, 1986.
7. Patten CJ, Ning SM, Lu AYH and Yang CS, Acetone-inducible cytochrome P-450: Purification, catalytic activity and interaction with cytochrome b_5. *Arch Biochem Biophys* **251**: 629–638, 1986.
8. Johansson I, Ekström G, Sholte B, Puzycki D, Jörnvall H and Ingelman-Sundberg M, Ethanol-, fasting-, and acetone-inducible cytochromes P-450 in rat liver: Regulation and characteristics of enzymes belonging to the IIB and IIE gene subfamilies. *Biochemistry* **27**: 1925–1934, 1988.
9. Ingelman-Sundberg M and Hagbjörk A-L, On the significance of the cytochrome P-450-dependent hydroxyl radical-mediated oxygenation mechanism. *Xenobiotica* **12**: 673–686, 1982.
10. Ingelman-Sundberg M and Johansson I, Mechanisms of hydroxyl radical formation and ethanol oxidation by ethanol-inducible and other forms of rabbit liver microsomal cytochromes P-450. *J Biol Chem* **259**: 6447–6458, 1984.
11. Ingelman-Sundberg M and Jörnvall H, Induction of the ethanol-inducible form of rabbit liver microsomal cytochrome P-450 by inhibitors of alcohol dehydrogenase. *Biochem Biophys Res Commun* **124**: 375–382, 1984.
12. Sinclair J, Cornell NW, Zaitlin L and Hansch C, Induction of cytochrome P-450 by alcohols and 4-substituted pyrazoles. Comparison of structure–activity relationships. *Biochem Pharmacol* **35**: 707–710, 1986.
13. Murray M, Wilkinson CF, Marcus C and Dubé CE, Structure–activity relationships in the interactions of alkoxymethylenedioxybenzene derivatives with rat hepatic microsomal mixed-function oxidases *in vivo*. *Mol Pharmacol* **24**: 129–136, 1983.
14. Marcus CB, Wilson NM, Jefcoate CR, Wilkinson CF and Omiecinski CJ, Selective induction of cytochrome P-450 isozymes in rat liver by 4-*n*-alkyl-methylenedioxybenzenes. *Arch Biochem Biophys* **277**: 8–16, 1990.
15. Giger U and Meyer UA, The substituted pyridines metyrapone and nicotinamide are inducers of 5-aminolevulinate synthase and cytochrome P-450 in hepatocyte culture. *Biochem Pharmacol* **31**: 1735–1741, 1982.
16. Funae Y and Imaoka S, Simultaneous purification of multiple forms of rat liver microsomal cytochrome P-450 by high-performance liquid chromatography. *Biochim Biophys Acta* **842**: 119–132, 1985.
17. Imaoka S, Terano Y and Funae Y, Purification and characterization of two constitutive cytochromes P-450 (F-1 and F-2) from adult female rats: Identification of P-450F-1 as the phenobarbital-inducible cytochrome P-450 in male rat liver. *Biochim Biophys Acta* **916**: 358–367, 1987.
18. Imaoka S, Terano Y and Funae Y, Changes in the

amount of cytochrome P450s in rat hepatic microsomes with starvation. *Arch Biochem Biophys* **278**: 168–178, 1990.
19. Imaoka S, Terano Y and Funae Y, Constitutive testosterone 6β-hydroxylase in rat liver. *J Biochem (Tokyo)* **104**: 481–487, 1988.
20. Nebert DW, Nelson DR, Coon MJ, Estabrook RW, Feyereisen R, Fujii-Kuriyama Y, Gonzalez FJ, Guengerich FP, Gunsalus IC, Johnson EF, Loper JC, Sato R, Waterman MR and Waxman DJ, The P450 superfamily: Update on new sequences, gene mapping, and recommended nomenclature. *DNA Cell Biol* **10**: 1–14, 1991.
21. Kamataki T, Maeda K, Yamazoe Y, Nagai T and Kato R, Sex difference of cytochrome P-450 in the rat: Purification, characterization, and quantitation of constitutive forms of cytochrome P-450 from liver microsomes of male and female rats. *Arch Biochem Biophys* **225**: 758–770, 1983.
22. Kato R, Yamazoe Y, Shimada M, Murayama N and Kamataki T, Effect of growth hormone and ectopic transplantation of pituitary gland on sex-specific forms of cytochrome P-450 and testosterone and drug oxidations in rat liver. *J Biochem (Tokyo)* **100**: 895–902, 1986.
23. Bandiera S, Ryan DE, Levin W and Thomas PE, Age- and sex-related expression of cytochromes P450f and P450g in rat liver. *Arch Biochem Biophys* **248**: 658–676, 1986.
24. Thummel KE, Favreau LV, Mole JE and Schenkman JB, Further characterization of RLM2 and comparison with a related form of cytochrome P-450, RLM2b. *Arch Biochem Biophys* **266**: 319–333, 1988.
25. Imaoka S, Terano Y and Funae Y, Expression of four phenobarbital-inducible cytochrome P-450s in liver, kidney and lung of rats. *J Biochem (Tokyo)* **105**: 939–945, 1989.
26. Hansch C and Leo A, *Substituents for Correlation Analysis in Chemistry and Biology*. Wiley-Interscience, New York, 1979.
27. Faureau LV, Malchoff DM, Mole JE and Schenkman JB, Responses to insulin by two forms of rat hepatic microsomal cytochrome P-450 that undergo major (RLM6) and minor (RLM5b) elevations in diabetes. *J Biol Chem* **262**: 14319–14326, 1987.
28. Imaoka S, Shimojo N and Funae Y, Induction of renal cytochrome P-450 in hepatic microsomes of diabetic rats. *Biochem Biophys Res Commun* **152**: 680–687, 1988.
29. Aoyama T, Hardwick JP, Imaoka S, Funae Y, Gelboin HV and Gonzalez FJ, Clofibrate-inducible rat hepatic P450s IVA1 and IVA3 catalyze the ω- and (ω-1)-hydroxylation of fatty acids and the ω-hydroxylation of prostaglandins E_1 and $F_{2\alpha}$. *J Lipid Res* **31**: 1477–1482, 1990.
30. Levin W, Thomas PE, Ryan DE and Wood AW, Isozyme specificity of testosterone 7α-hydroxylation in rat hepatic microsomes: Is cytochrome P-450a the sole catalyst? *Arch Biochem Biophys* **258**: 630–635, 1987.
31. Song B-J, Gelboin HV, Park S-S, Yang CS and Gonzalez FJ, Complementary DNA and protein sequences of ethanol-inducible rat and human cytochrome P-450s. Transcriptional and post-transcriptional regulation of the rat enzyme. *J Biol Chem* **261**: 16689–16697, 1986.
32. Fonné R and Meyer UA, Mechanisms of phenobarbital-type induction of cytochrome P-450 isozymes. *Pharmacol Ther* **33**: 19–22, 1987.