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CYTOCHROME P-450-MEDIATED METABOLISM OF BIPHENYL AND THE 4-HALOBIPHENYLS

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Abstract—The *in vitro* metabolism of biphenyl, 4-fluoro-, 4-chloro-, 4-bromo- and 4-iodobiphenyl by cytochrome P-450-dependent monooxygenases was investigated using hepatic microsomes from immature male rats pretreated with phenobarbitone or 3-methylcholanthrene. The major route of metabolism of biphenyl and the 4-halobiphenyls was 4'-hydroxylation, i.e. *para* to the phenyl–phenyl bridge. The minor route of metabolism apparently changed from 2-hydroxylation for biphenyl (i.e. *ortho* to the phenyl–phenyl bridge) to 3-hydroxylation for all 4-halobiphenyls (i.e. *ortho* to the halogen). In marked contrast to biphenyl, the regioselectivity of 4-halobiphenyl metabolism was not altered by pretreatment of rats with either phenobarbitone or 3-methylcholanthrene. The overall rate of metabolism of 4-fluoro- and 4-bromobiphenyl to water-soluble metabolites increased 2-fold and 5- to 6-fold using microsomes from rats pretreated with phenobarbitone and 3-methylcholanthrene respectively. In contrast, the overall rates of metabolism of 4-chloro- and 4-iodobiphenyl were refractory to the inductive effects of phenobarbitone but were increased more than 10-fold following pretreatment with 3-methylcholanthrene. There was a correlation between the apparent binding affinities of microsomes from phenobarbitone-treated rats for biphenyl and the 4-halobiphenyls and their calculated log octanol/water partition coefficients (lipophilicity). However, the effects of the halogen substituents on the rates of metabolism of the 4-halobiphenyls by microsomes from control and induced rats did not correlate with their binding affinities or with any physiochemical differences between the fluoro, chloro, bromo and iodo substituents.

The cytochrome P-450 inducers phenobarbitone (PB) and 3-methylcholanthrene (MC) exert different effects on the regioselective hydroxylation of biphenyl by rat liver microsomes [1–5]. The rate of 3- or 4-hydroxylation of biphenyl catalysed by hepatic microsomes from PB-treated rats is several-fold greater than that catalysed by microsomes from MC-treated rats, whereas the latter are several-fold more active in supporting the 2-hydroxylation of biphenyl. When chlorobenzene is used as substrate, a similar differential distribution of 2-, 3- and 4-hydroxylase activity is observed [6].

Like biphenyl and chlorobenzene, 4-chlorobiphenyl is hydroxylated principally in the *para* position to give 4'-chloro-4-biphenylol [7]. However, in contrast to biphenyl and chlorobenzene, the regiospecificity of the hydroxylation of 4-chlorobiphenyl by hepatic microsomes is unaffected by pretreatment of rats with PB or MC [8–10]. Furthermore, the rate of 4-chlorobiphenyl metabolism is markedly increased by MC treatment whereas PB treatment exerts little or no effect. Thus, both the rate and preferential site of hydroxylation of biphenyl and 4-chlorobiphenyl differ with respect to the effects of PB and MC treatment.

In light of the unexpected effects of the 4-chloro substituent on the microsomal enzyme-mediated metabolism of 4-chlorobiphenyl, the comparative metabolism of biphenyl, 4-fluoro-, 4-chloro-, 4-bromo- and 4-iodobiphenyl by hepatic microsomes from rats pretreated with PB or MC has been investigated.

MATERIALS AND METHODS

Glucose-6-phosphate, glucose-6-phosphate dehydrogenase (Baker's yeast), 3-methylcholanthrene, and NADP were purchased from the Sigma Chemical Co., St. Louis, MO. Sodium phenobarbitone was supplied by the Ontario Veterinary College, Guelph. Tritiated samples of biphenyl and the 4-halobiphenyls were prepared by the New England Nuclear Corp., Boston, MA, and purified by thin-layer chromatography (TLC) on 0.8 mm silicic acid G-UV_{254} (Merck) developed in hexane. To remove any contaminating oxidation products, each substrate was dissolved in hexane, washed with aqueous NaOH (0.5 M), and subjected to Florisil column chromatography using hexane as eluant. The radiochemical purity of the substrates was >99% as determined by TLC analysis, and the chemical purity was >99% as determined by gas–liquid chromatography (GLC) analysis using a flame ionization detector. The specific activity of each substrate was as follows: biphenyl, 11.0 Ci/mole; 4-fluorobiphenyl, 12.1 Ci/mole; 4-chlorobiphenyl, 9.7 Ci/mole; 4-bromobiphenyl, 10.3 Ci/mole; and 4-iodobiphenyl, 5.6 Ci/mole.

Animal treatment and isolation of microsomes. One-month-old male Wistar albino rats, average weight 100 g, were housed in wire cages and allowed

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free access to Purina Rat Chow and water. Phenobarbitone, dissolved in isotonic saline, was injected i.p. into twelve rats (400 μmoles/kg) on three consecutive days. 3-Methylcholanthrene, dissolved in corn oil, was injected i.p. into twelve rats (100 μmoles/kg) on two consecutive days. Fifteen control rats received a corresponding volume of corn oil (5 ml/kg) on two successive days. After the last injection, the rats were starved for 24 hr to lower liver glycogen levels and were killed by cervical dislocation.

Each liver was perfused via the hepatic portal vein with ice-cold isotonic saline supplemented with EDTA (0.1 mM). Two or three livers were combined and homogenised in sucrose–EDTA (0.25 M–0.1 mM). The microsomal fraction was harvested by differential centrifugation essentially as described [11].

Within each group, all microsomal fractions were combined and resuspended in sucrose–EDTA solution to a final protein concentration of 3 mg/ml as determined by the method of Lowry *et al.* [12] using bovine serum albumin as the standard. Various aliquots intended for metabolism studies were stored frozen at −20° and used within 3 weeks during which no loss of cytochrome P-450-dependent enzymic activity was detected. It has been shown that, unlike enzymic activity, the spectrally apparent interaction between chemical and oxidised microsomes may be affected by storage of the microsomes [13]. For this reason, such studies were performed immediately on freshly prepared microsomal suspensions.

Identification of metabolites. The metabolites of biphenyl, 4-chloro- and 4-bromobiphenyl formed both *in vivo* and *in vitro* have been described [1, 7, 14, 15]. Although the metabolites of 4-fluorobiphenyl formed *in vitro* have been studied [16], the products of the *in vitro* metabolism of 4-fluoro- and 4-iodobiphenyl have not. These products were characterised following the metabolism of non-radiolabelled 4-fluoro- and 4-iodobiphenyl (1 mM) for 90 min in large-scale incubation mixtures (20 times the incubation mixture described below) containing 90 mg hepatic microsomal protein from rats treated simultaneously with PB and MC. After acidification with acetate buffer (1.0 M, pH 4.0), incubation mixtures were extracted with diethyl ether (5 × 25 ml) and the combined extracts were evaporated to dryness.

The residue was redissolved in acetone and methylated by refluxing with methyl iodide and anhydrous potassium carbonate. The reaction mixture was filtered, concentrated to dryness, redissolved in petroleum ether, and filtered through a short Florisil column. The methyl ether metabolites were eluted with petroleum ether–diethyl ether (47:3).

Gas chromatography–mass spectrometry (GC-MS) of the methyl ether metabolites was determined on a VG Micromass 7070 high resolution mass spectrometer at 70 eV in the EI mode. GC analysis of the methyl ethers was carried out using a column packed with Ultrabonded Carbowax 20 M (RFR Corp.) as described [17].

Metabolism studies. The *in vitro* metabolism of biphenyl and the 4-halobiphenyls was performed under aerobic conditions in 3-ml incubation mixtures with 1 mg microsomal protein per ml of Tris·HCl buffer (17 mM, pH 8.0) containing $MgCl_2$ (1.0 mM), NADP (0.5 mM), D-glucose-6-phosphate (4.5 mM) and D-glucose-6-phosphate dehydrogenase (1.0 unit/ml), at the final concentrations indicated. Samples were preincubated at 37° in a shaking water bath (100 oscillations/min) for 15 min to generate NADPH. The reactions were initiated (usually at 15-sec intervals) by the addition of substrate in 100 μl methanol–Tween 80 (95:5, v/v) to a final concentration of 100 μM.

Reactions were stopped after 10 min with 3 ml NaOH (0.5 M) in ethanol (80%), and the metabolites were separated from unreacted starting material by a differential extraction procedure. The unreacted substrate was extracted (>99%) with 2 × 5 ml hexane as described for benzo[*a*]pyrene [18] and 4-chlorobiphenyl [8]. Less than 1% of the metabolites co-extracted with the unreacted substrate as previously reported for 4-chlorobiphenyl [8]. The metabolites (containing non-detectable amounts of unreacted substrate) were quantified by determining the radioactivity in an aliquot (0.5 or 1.0 ml) of the aqueous phase after acidification with HCl. The background radioactivity determined in zero-time incubations was subtracted from each sample.

After hexane extraction (and removal of an aliquot for radiocounting), the incubation mixtures were acidified with HCl and extracted four times with equal volumes of diethyl ether. The combined ether extracts were allowed to evaporate to approximately 1 ml which was analysed by TLC on 0.75 mm silicic acid HF_{254} (BDH). For biphenyl, the developing medium was benzene–ethanol (95:5, v/v), whereas chloroform–acetic acid (99:1, v/v) was used for each of the 4-halobiphenyls. Each plate was divided into twenty parts which were scraped into liquid scintillation vials. The small amount of silicic acid was found not to interfere with the determination of radioactivity. Samples were kept in the dark for 24 hr to avoid chemiluminescence. The background radioactivity determined in zero-time incubations was subtracted from each sample.

Binding to cytochrome P-450. The putative binding of biphenyl and the 4-halobiphenyls to cytochrome P-450 was examined by room temperature difference spectrophotometry essentially as described [19]. Freshly prepared microsomes were diluted to a final protein concentration of 1.0 mg/ml with phosphate buffer (0.1 M, pH 7.4), and 3 ml was added to each of two matched quartz cuvettes. After a baseline of equal light absorbance was established, increasing amounts of each substrate were added using dimethylsulphoxide (DMSO) as the solvent. DMSO was chosen because, unlike many other solvents, it fails to cause any spectrally apparent interaction with oxidised microsomes between 350 and 500 nm [20]. For this reason, a corresponding volume of DMSO was not added to the reference cuvette.

Microsomal suspensions were scanned between 350 and 500 nm in the presence of 0, 1, 2, 3, 4, 5, 7 and 10 μM substrate. Spectra were recorded on a Cary 118C spectrophotometer equipped with a repetitive scan accessory and calibrated with a holmium oxide crystal.

Statistics. The statistical significance of the difference between the noninduced and the induced rates of metabolism was analysed by Student's *t*-test at the 1% level of significance.

RESULTS

Characterisation of 4-fluoro- and 4-iodobiphenyl metabolites. GC–MS analysis of the methyl ether derivatives of the 4-fluorobiphenyl metabolites indicated the formation of two 4-fluorobiphenylol methyl ethers (62%) (retention time 6.20 and 7.85 min) and one 4-fluorobiphenyldiol dimethyl ether (38%) (retention time 11.37 min). The fragmentation pattern of the major monohydroxylated methyl ether metabolite (58%) (retention time 7.85 min) demonstrated intense molecular M^+. (*m/e* 202) and M^+.-15 (*m/e* 187) ions which were characteristic of a *para*-substituted methoxyl group [21] and is consistent with the 4'-fluoro-4-biphenylol methyl ether structure. The identities of the other two metabolites could not be unequivocally assigned. Comparable GC–MS of the 4-iodobiphenyl metabolites confirmed the formation of two monohydroxy (84%) (retention times: 7.37 and 7.83 min) and three dihydroxy (16%) metabolites (retention times: 8.88, 9.33 and 10.53 min). The fragmentation pattern of the major 4-iodobiphenylol methyl ether metabolite (58%) (retention time: 7.83 min) also exhibited intense M^+ (*m/e* 310) and M^+.-15 (*m/e* 295) ions consistent with the 4'-iodo-4-biphenylol methyl ether structure [21]. The fragmentation patterns of the remaining four methyl ether metabolites did not permit the unambiguous assignment of their structures.

It is important to note that, in order to generate metabolites for structural characterisation, 4-fluoro- and 4-iodobiphenyl were incubated in large-scale incubation mixtures for 90 min (see Materials and Methods). Under such conditions, secondary metabolism of primary metabolites is likely. Therefore, the ratio of monohydroxylated to dihydroxylated metabolites generated in the large-scale incubations should not be compared to the ratio of metabolites generated under incubation conditions (see below) in which linear relations with respect to protein concentration and time were established.

Effects of induction on rates of metabolism. The overall rates of metabolism of biphenyl and the 4-halobiphenyls to water-soluble metabolites by hepatic microsomes from control, PB-treated and MC-treated rats are shown in Table 1. Biphenyl, 4-fluoro- and 4-iodobiphenyl were metabolised by control microsomes at approximately equal rates ($\sim$70 pmoles·(mg protein)$^{-1}$·min^{-1}). 4-Chlorobiphenyl and 4-bromobiphenyl were metabolised at twice and half this rate respectively. The reason for this variation is unknown although a similar difference between biphenyl and 4-chlorobiphenyl has been reported previously [22].

We are unable to explain why the rates of biphenyl and 4-chlorobiphenyl metabolism are lower than those reported by others [1–5, 10, 23]. The rate of metabolism of 4-chlorobiphenyl by control rat liver microsomes reported by Wolff and Hesse [23] is twenty times greater than the rate shown in Table 1. However, the rate of aminopyrine *N*-demethylation reported by the same authors is ten to twenty times less than the rate routinely observed in our laboratory (e.g. Ref. 8). It is possible that such differences in basal metabolic activity reflect strain or colony differences in rats.

On the basis of their responses to PB and MC treatment, the five substrates could be categorised into two groups. The rate of metabolism of the first group, comprising biphenyl, 4-fluoro- and 4-bromobiphenyl, was increased approximately 2-fold and 5- to 6-fold by PB and MC treatment respectively. The rate of metabolism of the second group, comprising 4-chloro- and 4-iodobiphenyl, was not increased significantly by PB treatment but was increased by more than an order of magnitude by MC treatment.

Analysis of metabolite profiles. The TLC distribution patterns of the ether-extractable metabolites of each 4-halobiphenyl were very similar, with three regions of radioactivity apparent at R_f 0–0.2, R_f

Table 1. Effects of phenobarbitone (PB) and 3-methylcholanthrene (MC) treatment on the overall rate of metabolism of biphenyl and the 4-halobiphenyls to total water-soluble metabolites*

	Rate of metabolism		
	Microsomal preparation		
Substrate	Noninduced	PB-induced	MC-induced
Biphenyl	72.8 ± 6.3	138 ± 9† (1.9)	433 ± 22† (5.9)
4-Fluorobiphenyl	74.1 ± 6.1	149 ± 11† (2.0)	392 ± 19† (5.3)
4-Chlorobiphenyl	129 ± 8	137 ± 14 (1.1)	1590 ± 80† (12.3)
4-Bromobiphenyl	29.9 ± 4.1	53.1 ± 3.1† (1.8)	172 ± 10† (5.8)
4-Iodobiphenyl	60.7 ± 3.9	61.8 ± 5.3 (1.0)	1400 ± 120† (23.1)

* Values are mean pmoles metabolised·(mg protein)$^{-1}$·min^{-1} ± S.D. Numbers in parentheses = induced rate/noninduced rate.

† Significantly increased over noninduced rate of metabolism ($P < 0.01$).

0.4–0.6 and R_f 0.75–0.85. The absence of any radioactivity at R_f 0.9–1.0 indicated that all unreacted substrate had been successfully removed by prior extraction with hexane. For each 4-halobiphenyl, between 90 and 97% of the total radioactivity associated with the metabolites was located in the region R_f 0.4–0.6, irrespective of the animal pretreatment. This indicated that the oxidation site specificity was not altered by either PB or MC treatment. Previous studies with 4-chlorobiphenyl [19] and 4-bromobiphenyl [11] and the data presented above for 4-fluorobiphenyl and 4-iodobiphenyl suggest that this major metabolite, produced by both cytochrome P-450- and cytochrome P-448-dependent monooxygenases, was 4′-halo-4-biphenylol which corresponds to the GC–MS analysis of the metabolite methyl ether derivatives. For 4-chlorobiphenyl, the minor metabolite (*ca.* 5% of the total radioactivity) at R_f 0.75 corresponds to 4-chloro-3-biphenylol [15]. Based on their chromatographic behaviour, the minor metabolite of the other 4-halobiphenyls (2.5–8% of the total radioactivity) at R_f 0.75–0.85 was presumably 4-halo-3-biphenylol while dihydroxylated metabolites probably accounted for the small

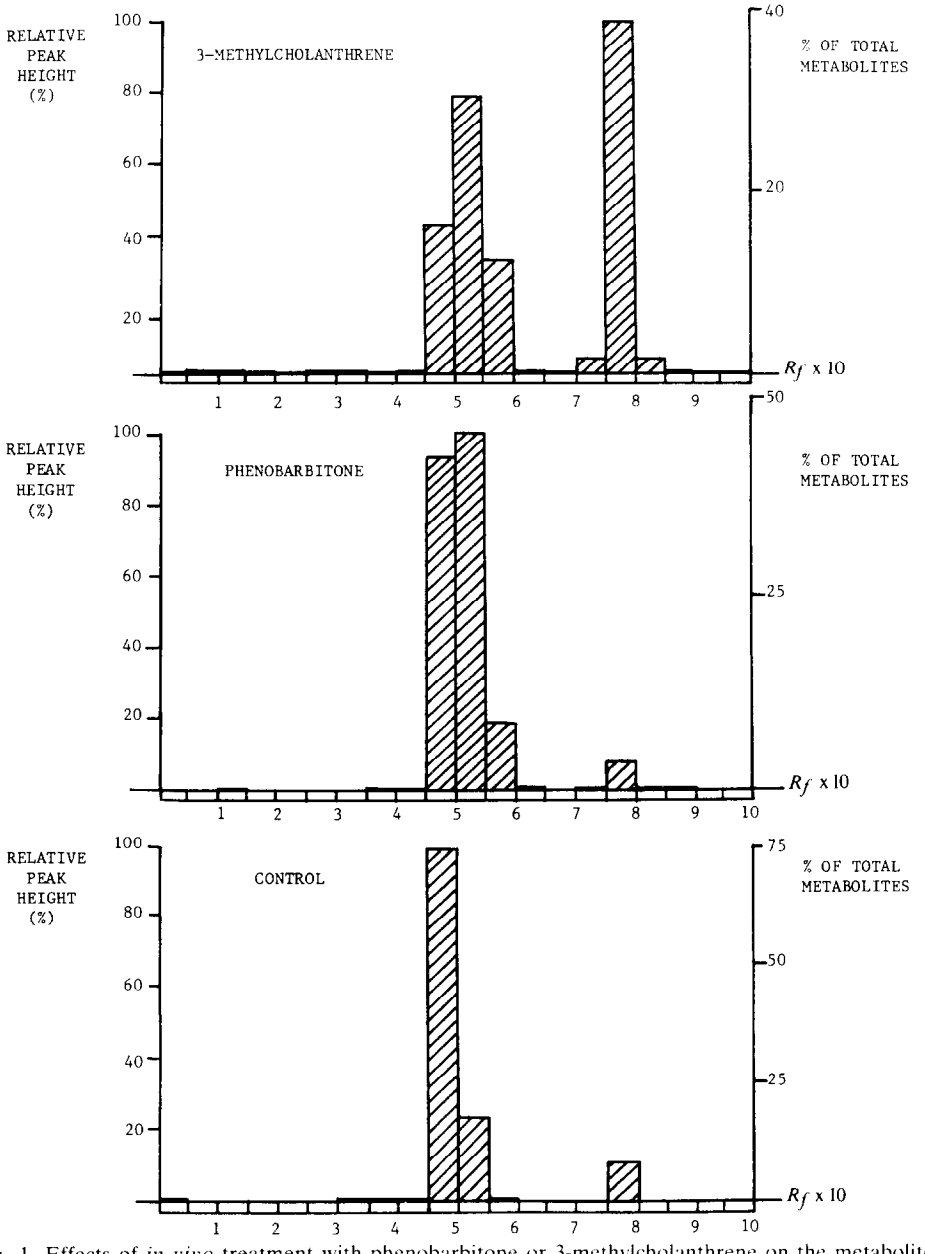

Fig. 1. Effects of *in vivo* treatment with phenobarbitone or 3-methylcholanthrene on the metabolite profile (radio-thin-layer chromatogram) of biphenyl. Tritiated biphenyl (100 μM) was metabolised by microsomes from rats treated with corn oil (control) (bottom), phenobarbitone (centre) or 3-methylcholanthrene (top). Following the removal of unreacted biphenyl, the metabolites were chromatographed on silicic acid HF_{254} developed in benzene–ethanol (95:5, v/v) as described [15]. The radioactivity in each of twenty divisions is expressed either relative to the major peak designated 100% (left ordinate) or as a percent of the total radioactivity (right ordinate).

amount of radioactivity (0.5–8.0%) located near the origin.

In contrast to the 4-halobiphenyls, animal pretreatment markedly influenced the regioselectivity of biphenyl hydroxylation. The TLC analyses of the profile of metabolites formed using microsomes from control, PB-treated and MC-treated rats are illustrated in Fig. 1. Using microsomes from control and PB-treated rats, two major bands of radioactivity were located at R_f 0.5 and 0.78 which, based on their chromatographic mobilities [24] and comparison with authentic standards, were identified as 4-biphenylol and 2-biphenylol respectively. The ratio of the 4-hydroxy to 2-hydroxy metabolite was approximately 12 to 1 and 28 to 1 for microsomes from control and PB-treated rats respectively. In contrast to PB, pretreatment *in vivo* with MC markedly decreased the ratio of the 4-hydroxy to 2-hydroxy metabolite to about 1.4 to 1.

Figure 1 also illustrates two additional points. First, following treatment with PB or MC, a broadening of the radioactive band at R_f 0.5 was observed. The nature of this effect is consistent with the presence of either increased amounts of 4-biphenylol or small amounts of 3-biphenylol. Dihydroxylated metabolites of biphenyl probably account for the trace quantities of radioactivity between the origin and 4-biphenylol [24]. Second, although constitutive in the young, the activity of biphenyl 2-hydroxylase declines with age in both the rat and rabbit and is undetectable, but still inducible, in untreated adults [1, 25]. In view of this age-dependent variation, it should be noted that the formation of 2-biphenylol by control microsomes is consistent with the fact that the rats used in this study were immature (1-month old).

Binding to cytochrome P-450. The addition of biphenyl and the 4-halobiphenyls (dissolved in DMSO) to one of two matched cuvettes containing oxidised microsomes (1 mg protein/ml) produced, in all cases, a Type I spectrum with a trough at 419 nm and a peak at 387 nm. The magnitude of the absorbance change ($\Delta A = A_{500} - A_{419}$) following addition of each substrate (final concentration 20 μM) to microsomes from control, PB-induced and MC-induced rats is shown in Fig. 2. The results suggest that the magnitude of the absorbance change elicited by biphenyl and each 4-halobiphenyl paralleled the lipophilicity of these chemicals.

The values of ΔA/mg protein shown in Fig. 2 indicate that PB treatment slightly more than doubled the absorbance change elicited by each chemical. However, since PB also increased cytochrome P-450 content by 2.3-fold, the values of ΔA/nmole P-450 were essentially unaffected by PB treatment. In contrast, MC treatment decreased ΔA/mg protein for biphenyl, 4-fluoro- and 4-chlorobiphenyl and decreased ΔA/nmole P-450 for all chemicals except 4-iodobiphenyl (the cytochrome P-450 content was induced 2-fold by MC). Since the Type I spectrum

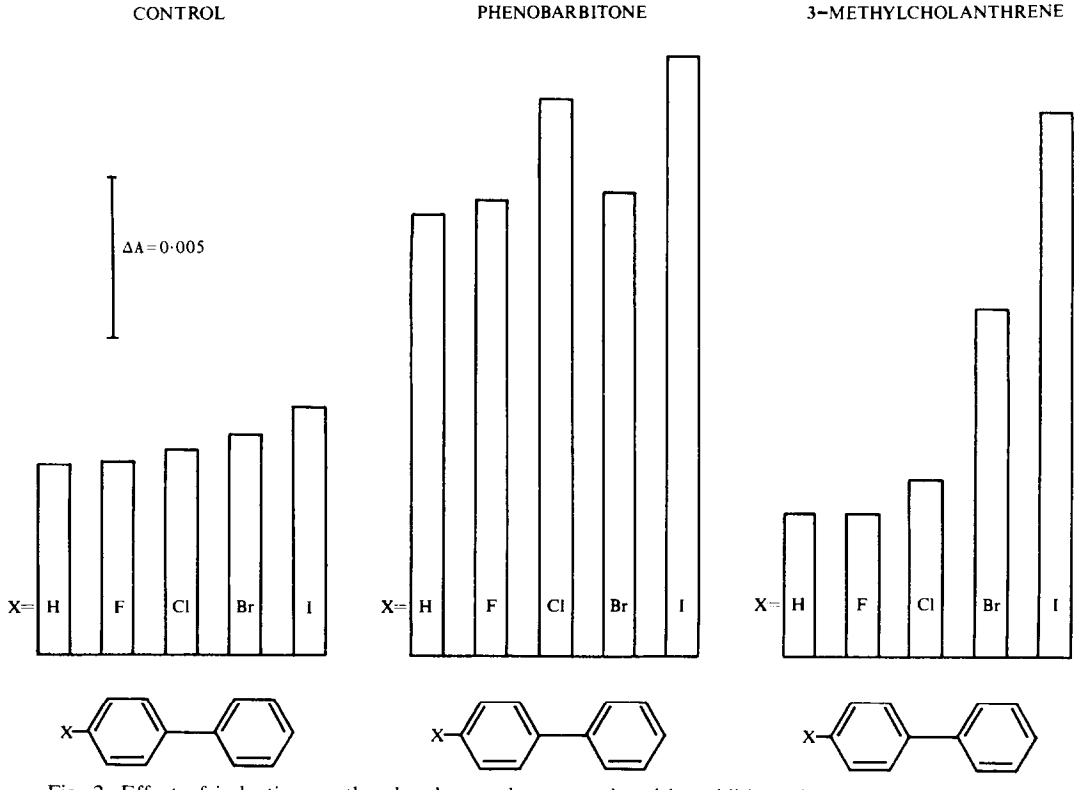

Fig. 2. Effect of induction on the absorbance change produced by addition of substrate (20 μM) to oxidised microsomes. Each chemical was added in 20 μl DMSO to hepatic microsomes from rats pretreated with corn oil (control), phenobarbitone or 3-methylcholanthrene (1 mg protein/ml phosphate buffer, pH 7.4), and the absorbance change (ΔA) was measured between A_{419} and A_{500}.

reflects the transition from low-spin to high-spin ferricytochrome P-450 [20], the attenuation of ΔA by MC treatment is consistent with reports that microsomes from MC-treated rats have a higher percentage of high-spin character than microsomes from either corn oil- or PB-treated rats [26, 27].

In Fig. 3, iodobiphenyl exemplifies the effect of adding increasing concentrations of each chemical to microsomes from PB-treated rats. Iodobiphenyl, dissolved in DMSO, was added to 1-μl aliquots (using a Hamilton microsyringe) at seven concentrations (1, 2, 3, 4, 5, 7 and 10 μM) labelled A–G. Throughout these titrations, DMSO was not added to the reference cuvette since addition of up to 20 μl to a 3-ml microsomal sample failed to elicit a spectrally apparent interaction between 350 and 500 nm as previously reported [20]. With increasing concentration, iodobiphenyl, like all substrates, caused an increasing absorbance change (measured between the trough at 419 nm and the isosbestic point, 500 nm).

A double-reciprocal plot of absorbance change (ΔA) versus substrate concentration ([S]) gave straight lines for the interaction of biphenyl and the 4-halobiphenyls with microsomes from PB-treated rats. Such plots (exemplified by 4-iodobiphenyl in Fig. 4) permitted estimation of the apparent spectral dissociation constant, K_s^{app} (defined as that substrate concentration causing half-maximum absorbance change).

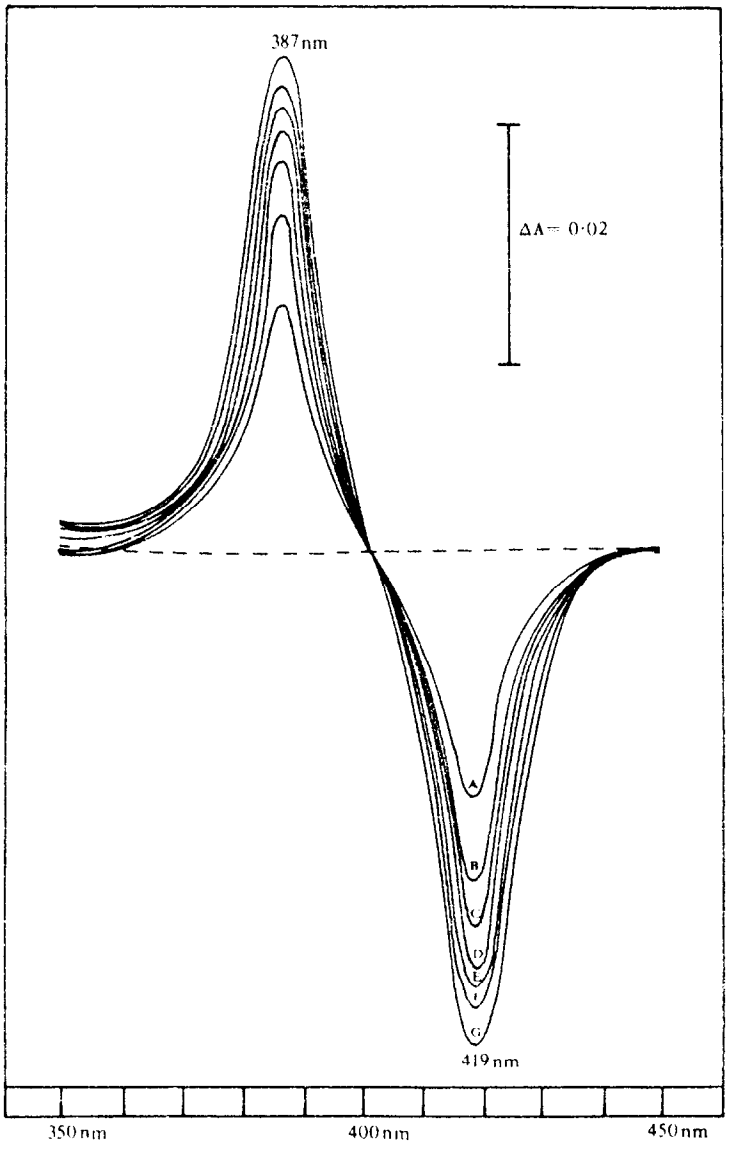

Fig. 3. Difference spectrum of iodobiphenyl interaction with microsomes from phenobarbitone-treated rats. Iodobiphenyl dissolved in DMSO was added to microsomes from phenobarbitone-treated rats (1 mg microsomal protein/ml phosphate buffer, pH 7.4) at the following final concentrations: 1, 2, 3, 4, 5, 7 and 10 μM designated A–G respectively. The dashed line is a baseline (no iodobiphenyl) in the presence or absence of 20 μl DMSO in the reference cuvette. Spectra were recorded using a Cary 118C spectrophotometer with a repetitive scan accessory and calibrated with holmium oxide.

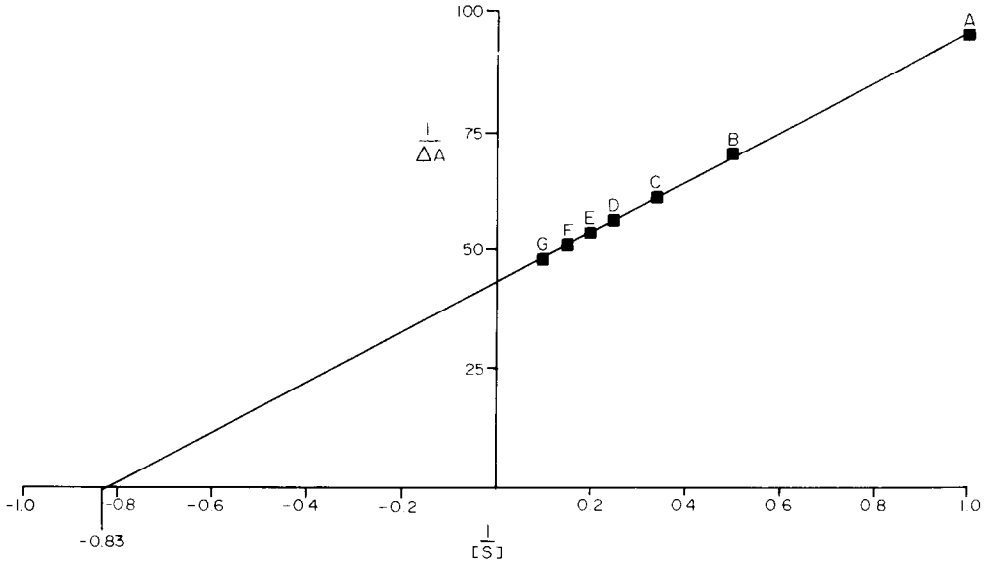

Fig. 4. Double-reciprocal plot of absorbance change (ΔA) versus iodobiphenyl concentration in microsomes from phenobarbitone-treated rats. The concentrations of iodobiphenyl (1, 2, 3, 4, 5, 7 and 10 μM) are labelled A–G in accordance with Fig. 3. The corresponding absorbance changes ($\Delta A = A_{419} - A_{500}$) are from the same figure.

Without increasing the concentration of microsomal protein, K_s^{app} values (which are dependent on protein concentration [20]) could not be determined using microsomes from corn oil- or MC-treated rats due to the low specific activity and higher percentage of high-spin ferricytochrome P-450 in these preparations respectively.

As shown in Fig. 5, the values of K_s^{app} using microsomes from PB-treated rats decreased with increasing substrate lipophilicity (taken as log P where P is the octanol:water partition coefficient). In other words, as their lipophilicity increased, the apparent affinity with which cytochrome P-450 bound each substrate increased.

DISCUSSION

The present study revealed a number of similarities and differences between biphenyl and the 4-halobiphenyls in their interactions with, and metabolism

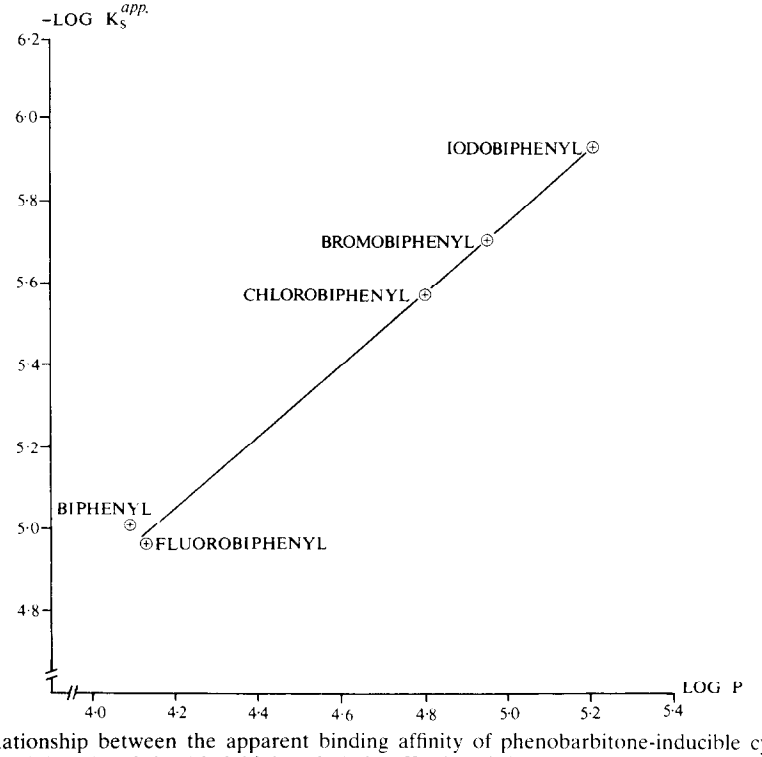

Fig. 5. Relationship between the apparent binding affinity of phenobarbitone-inducible cytochrome P-450 for biphenyl and the 4-halobiphenyls ($-\log K_s^{app}$) and the lipophilicity of these chemicals.

by, cytochrome P-450-dependent monooxygenases. Each chemical was qualitatively similar in that 4-hydroxylation was the major route of metabolism. In contrast, the minor route of metabolism apparently changed from 2-hydroxylation to 3-hydroxylation upon *para* halogenation of biphenyl, a change consistent in part with the *ortho/para*-directing effect of halogens. Biphenyl also differed from the 4-halobiphenyls with respect to oxidation site specificity in that the ratio of the rates of the minor to major pathway decreased following PB treatment and increased following MC treatment. However, such changes in regioselectivity were not observed for the 4-halobiphenyls and, irrespective of animal pretreatment, 4'-halo-4-biphenylol dominated (>90%) the metabolite profile of 4-fluoro-, 4-chloro-, 4-bromo- and 4-iodobiphenyl.

Para chlorination and iodination of biphenyl rendered the rate of metabolism refractory to the inductive effects of PB. Indeed, the rate of metabolism of 4-chloro- and 4-iodobiphenyl expressed on a per nmole cytochrome P-450 basis decreased approximately 50% following PB treatment. In contrast to PB, MC treatment caused more than a 10-fold increase in the rate of metabolism of these two halobiphenyls. It would appear that 4-iodobiphenyl shares many of the properties previously reported for 4-chlorobiphenyl [8–10].

The 2.3-fold increase in cytochrome P-450 induced by PB was accompanied by a comparable increase in the rate of metabolism of biphenyl, 4-fluoro- and 4-bromobiphenyl. The rates of metabolism of these same three substrates were increased 5- to 6-fold by MC treatment. These responses to enzyme induction suggest that the effects of *para* fluorination and bromination of biphenyl differ from those of *para* chlorination and iodination. Attempts to correlate this and other differences between substrates with various physiochemical properties (parachor, octanol: water partition coefficients [P] and substituent electronic parameters [Hammett constants]) were unsuccessful. Furthermore, the intermediacy of an arene oxide has been implicated in the metabolism of biphenyl [3], 4-fluorobiphenyl [16], 4-chlorobiphenyl [28] and 4-bromobiophenyl [14], enabling hydroxylation to proceed with little or no isotope effect (since C—H bonds are not broken during the rate-limiting oxidation step). This suggests that possible differences in the distribution of tritium about the biphenyl nucleus are unlikely to alter the rate of oxidation.

In an attempt to explain the various differences between the metabolism of biphenyl and the 4-halobiphenyls, the putative binding of these chemicals to cytochrome P-450 was examined by room temperature difference spectrophotometry. The term "putative binding" is used because the Type I spectrum produced by each chemical reflects a transition from low-spin to high-spin ferricytochrome P-450 and is not, therefore, a direct measure of cytochrome P-450:substrate complex formation. In contrast to their rates of metabolism, both the magnitude of the absorbance change and the apparent binding affinity of cytochrome P-450 for biphenyl and the 4-halobiphenyls increased with increasing substrate lipophilicity. These spectrally apparent interactions failed, therefore, to indicate any preferential binding of the substrates to microsomes from either control, PB-treated or MC-treated rats. Thus, the reason for the differential effects exerted by the halogens on the metabolism of the biphenyl nucleus remains unknown.

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