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Relationship between Chemical Structure and Activity. II.[1] Influences of Isomers in Dichlorobenzene, Trichlorobenzene, and Tetrachlorobenzene on the Activities of Drug-Metabolizing Enzymes[2]

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Hepatic constituents and the cytochrome contents in addition to activities of drug-metabolizing enzymes and δ-aminolevulinic acid (δ-ALA) synthetase were examined in rats treated with each isomer of dichlorobenzene (DCB), trichlorobenzene (TRCB) and tetrachlorobenzene (TECB) in an oral dose of 250 mg/kg once daily for 3 days.

1) In the studies on DCB-isomers, activities of aminopyrine demethylase and aniline hydroxylase were enhanced markedly by treatment with *m*-DCB, whereas cytochrome content was not altered significantly by treatment with any DCB-isomers. δ-ALA synthetase activity was enhanced 63, 32 and 42% by treatment with *o*-, *m*- and *p*-DCB, respectively, but these enhancement were not paralleled with the changes in cytochrome P-450 content.

2) In the administration of TRCB-isomers, enzyme activities were enhanced markedly by treatment with 1,2,4-TRCB. Cytochrome P-450 content was also increased by treatment with 1,2,4-TRCB, and this increase could be partly related with the enhancement of δ-ALA synthetase activity.

3) By treatment with all TECB-isomers, activity of aminopyrine demethylase was enhanced, whereas aniline hydroxylase was not altered. Cytochrome P-450 content was increased by treatment with all TECB-isomers.

4) Microsomal protein content was increased with all isomers of DCB, TRCB and TECB treatment. Microsomal Pi was increased markedly 36, 70 and 91% by treatment with *m*-DCB, 1,2,4-TRCB and 1,2,3,5-TECB, respectively. Hepatic glycogen content was decreased only by 1,2,3,5-TECB, but triglyceride content was not altered.

5) The spectral change induced by substrate-cytochrome P-450 binding was characterized by type I in each treatment, but conversion of the type occurred in treatment with 1,2,4,5-TECB in high concentration of 1 mM.

It has been reported[4] that when polychlorinated biphenyls consisting of a number of isomers penetrate through a living animals, they always exert many complicated effects on the body, that is, abnormality in the lipid and calcium metabolism, induction of the drug-metabolizing enzymes or malignant influences on the normal physiological substances. These facts will lead to the suggestion that different effects may result from the different interaction of each isomer and the body organs.

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2) A part of this work was presented at the 81st Meeting of Kyushu Branch, Pharmaceutical Society of Japan, Fukuoka, February 1973.
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In the preceding paper,[1] the present authors investigated the influences of chlorine-number in the chlorinated benzenes on rats by determining the content of cytochromes and the activities of drug-metabolizing enzymes in hepatic microsomes, in addition to the hepatic constituents and the activity of δ-aminolevulinic acid (δ-ALA) synthetase. In the present experiments, we have determined the effects of isomers of the chlorinated benzenes after the treatment of rats with various isomers of dichlorobenzene, trichlorobenzene and tetrachlorobenzene, furthermore, the relationship between the chemical structure and their biological activities had been also pursued.

Materials and Methods

The chlorinated benzenes were purchased from the following sources: *o*-dichlorobenzene (*o*-DCB), *m*-dichlorobenzene (*m*-DCB), 1,2,3-trichlorobenzene (1,2,3-TRCB), 1,2,4-trichlorobenzene (1,2,4-TRCB) and 1,3,5-trichlorobenzene (1,3,5-TRCB) from Nakarai Chemicals Co. Ltd., Kyoto, Japan; *p*-dichlorobenzene (*p*-DCB), 1,2,3,4-tetrachlorobenzene (1,2,3,4-TECB) and 1,2,4,5-tetrachlorobenzene (1,2,4,5-TECB) from Tokyo Kasei Kogyo Co. Ltd., Tokyo, Japan; 1,2,3,5-tetrachlorobenzene (1,2,3,5-TECB) from Schuchardt Co. Ltd., Muenchen, Germany. δ-ALA was purchased from Daiichi Pure Chemicals Co. Ltd., Tokyo, Japan. NADP, glucose 6-phosphate disodium salt (G-6-P), glucose 6-phosphate dehydrogenase (G-6-PD) were purchased from Boehringer Mannheim GmbH, Mannheim, Germany. Aniline and aminopyrine were obtained from commercial sources and used following purification by redistillation and recrystallization, respectively. The other reagents were obtained from commercial sources and used without further purification.

Female Wistar rats weighing 110—130 g were used in all experiments and they were fed commercial rat chow, F-II, Funahashi Nojyo Co. Ltd., Chiba, Japan, for one week prior to experiments.

Chlorinated benzenes suspended in 2% tragacanth gum solution were given to rats orally at a dose of 250 mg/5 ml/kg once daily for 3 days. The control animals received an equal volume of the vehicle.

Rats were killed by decapitation 24 hr after the last administration, and the livers were perfused with ice-cold 0.9% NaCl solution *in situ* to remove blood.

The livers were removed and weighed, and the portions of liver were immediately used for the estimation of glycogen[5] and triglyceride.[6] The rest of liver was immediately placed in ice-cold 0.9% NaCl solution. The livers were weighed and homogenized in 3 volumes of 0.25M sucrose solution containing 1 mM EDTA in a motordriven Potter homogenizer with a Teflon pestle. Preparation of microsomes was carried out by the procedures as described previously.[1,7] Microsomal protein was estimated by the method of Lowry, *et al.*,[8] using bovine serum albumin as a standard. Incubation mixtures and enzyme assays were carried out by the conditions as described previously.[1] *p*-Aminophenol formed by aniline hydroxylase was determined by the method of Imai and Sato[9] and formaldehyde formed by aminopyrine demethylase was determined according to Nash reaction.[10] Assay of cytochromes was estimated by the method of Omura and Sato.[11] Substrate-induced spectral change was obtained by the method of Schenkman, *et al.*[12] Inorganic phosphorus (Pi) analysis was carried out by the method of Fiske and Subbarow.[13] The activity of δ-ALA synthetase was incubated as described by Marver, *et al.*[14] and determined by the method of Urata and Granick.[15]

Results

Effects of DCB-isomers

The effects of treatment of rats with DCB-isomers in an oral dose of 250 mg/kg once daily for 3 days were shown in Table I. The ratio of liver weight to 100 g body weight was

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Table I. Effects of Treatment with Dichlorobenzene Isomers for 3 Days on Hepatic Constituents, Cytochromes, Drug-Metabolizing Enzymes and δ-Aminolevulinic Acid Synthetase

	Control	*o*-DCB	*m*-DCB	*p*-DCB
Body weight initial (g)	115±3	121±2	120±3	119±2
final (g)	127±3	130±2	129±2	129±1
Liver weight (g/100 g b.w.)	5.04± 0.06	6.26± 0.15[a]	6.10± 0.27[a]	5.41± 0.19
Glycogen (mg/g liver)	72.3 ± 3.0	81.5 ± 3.8	66.1 ± 3.6	69.5 ± 4.6
Triglyceride (mg/g liver)	6.1 ± 0.7	6.1 ± 0.7	5.7 ± 0.4	6.3 ± 0.5
Microsomal phosphorus (μg/g liver)	218.4 ±13.8	270.9 ±10.6	298.2 ±11.6[b]	230.1 ±27.8
protein (mg/g liver)	16.0 ± 0.4	17.7 ± 0.5[b]	19.2 ± 0.6[a]	19.6 ± 0.2[a]
Cytochromes				
P-450 (nmoles/mg protein)	0.69± 0.04	0.66± 0.03	0.77± 0.04	0.68± 0.01
b_5 (nmoles/mg protein)	0.32± 0.01	0.37± 0.02	0.36± 0.01	0.34± 0.01
Aniline hydroxylase				
formed *p*-aminophenol (nmoles/mg protein/min)	0.51± 0.03	0.57± 0.04	0.69± 0.04[a]	0.58± 0.04
formed *p*-aminophenol/P-450 (nmoles formed/nmoles P-450/min)	0.74± 0.07	0.86± 0.03	0.90± 0.3	0.85± 0.05
Aminopyrine demethylase				
formed formaldehyde (nmoles/mg protein/min)	4.55± 0.12	5.94± 0.38[b]	7.00± 0.60[a]	5.23± 0.31
formed formaldehyde/P-450 (nmoles formed/nmoles P-450/min)	6.60± 0.37	9.00± 0.52[a]	9.10± 0.72[a]	7.70± 0.43
Spectral change aniline-cytochrome P-450 ($E_{430-480}$/mg protein) × 10^3	15.5 ± 0.6	16.8 ± 0.9	16.6 ± 1.1	13.6 ± 0.4
δ-ALA synthetase (nmoles/g liver/hr)	22.6 ± 1.6	36.8 ± 3.0[a]	29.8 ± 0.7[b]	32.3 ± 3.9

Rats were pretreated orally with *o*-, *m*-, and *p*-DCB in a dose of 250 mg/kg once daily for 3 days, and were sacrificed 24 hr after the last administration. All values are mean ± S.E. of 6 rats except phospholipids value is mean ± S.E. of 3 groups of 2 rats.

a) significantly different from control, $p<0.01$

b) significantly different from control, $p<0.05$

increased by *o*- and *m*-DCB treatment, whereas glycogen and triglyceride contents were not affected by treatment with any DCB-isomers. Pi content in microsomal phospholipid was increased markedly by the treatment with all DCB-isomers. A significant increase was noted in microsomal protein content, but no change in cytochrome P-450 content. Moreover, cytochrome b_5 content was increased only to a slight extent, but not significant, that is, 11%, 11% and 6% by the treatment with *o*-DCB, *m*-DCB and *p*-DCB, respectively.

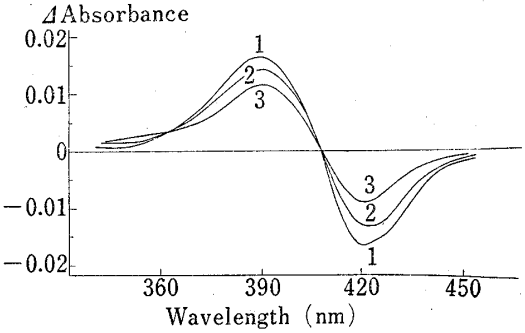

Fig. 1. Spectral Change of Dichlorobenzene-Isomers with Rat Liver Microsomes

Each cuvette contained in 3 ml: microsomal suspension (6 mg of protein)and 1.15% KCl-25 mM Tris-HCl, pH 7.4. The respective substrate of 1 mM was added in acetone to the sample cuvette and an equivalent amount of the acetone was added to the reference cuvette. The spectral dissociation constant (*K*s) was determined graphically from the X intercept of a double inverse plot of Δ absorbance at 420—500 nm *vs.* substrate concentration. 1: *o*-DCB, *K*s=0.25 mM; 2: *m*-DCB, *K*s=0.56 mM; 3: *p*-DCB, *K*s=0.77 mM.

On the other hand, activity of aminopyrine demethylase was enhanced by each treatment with *o*-DCB and *m*-DCB, and activity of aniline hydroxylase was enhanced only by the treatment with *m*-DCB. However, when the enzyme activity based on microsomal protein was calculated into that based on cytochrome P-450, no difference was noted in activity of aniline hydroxylase as compared to the control value, whereas activity of aminopyrine demethylase was enhanced by the treatment with *o*-DCB and *m*-DCB. This enhancement was large

as compared to the change in cytochrome P-450 content. δ-ALA synthetase activity was increased 63%, 32% and 43% by the treatment with *o*-DCB, *m*-DCB and *p*-DCB, respectively, but this enhancement was not parallel to the change in cytochrome content.

No significant difference was noted in the magnitude of aniline-cytochrome P-450 binding spectrum by the treatment with any isomers. The difference spectra induced by each DCB isomer-cytochrome P-450 binding were recorded with a maximum at 388—392 nm and a minimum at 419—422 nm, and thus they characterized by type I, as shown in Fig. 1.

Table II. Effects of Treatment with Trichlorobenzene Isomers for 3 Days on Hepatic Constituents, Cytochromes, Drug-Metabolizing Enzymes and δ-Aminolevulinic Acid Synthetase

	Control	1,2,3-TRCB	1,2,4-TRCB	1,3,5-TRCB
Body weight initial (g)	118±3	122±2	120±3	121±2
final (g)	132±3	134±1	128±3	131±2
Liver weight (g/100 g b.w.)	5.22± 0.20	5.63± 0.12	7.23± 0.21[a]	5.86± 0.26
Glycogen (mg/g liver)	70.1 ± 2.2	69.3 ± 0.12	66.5 ± 4.5	59.7 ± 4.6
Triglyceride (mg/g liver)	5.7 ± 0.6	5.0 ± 0.9	6.0 ± 0.3	7.6 ± 1.0
Microsomal phosphorus (μg/g liver)	238.0 ± 9.9	299.0 ±12.6[b]	404.4 ±20.6[a]	296·6 ±14.8[b]
protein (mg/g liver)	16.9 ± 0.3	18.3 ± 0.5[b]	21.6 ± 0.5[a]	21.0 ± 0.7[a]
Cytochromes				
P-450 (nmoles/mg protein)	0.70± 0.03	0.84± 0.02[b]	1.63± 0.06[a]	0.78± 0.03
b_5 (nmoles/mg protein)	0.32± 0.01	0.38± 0.02	0.36± 0.03	0.37± 0.02
Aniline hydroxylase				
formed *p*-aminophenol (nmoles/mg protein/min)	0.56± 0.03	0.65± 0.03	0.99± 0.03[a]	0.60± 0.04
formed *p*-aminophenol/P-450 (nmoles formed/nmoles P-450/min)	0.80± 0.04	0.77± 0.05	0.61±0.02[b]	0.77± 0.03
Aminopyrine demethylase				
formed formaldehyde (nmoles/mg protein/min)	2.90± 0.21	3.99± 0.45	11.58± 0.43[a]	4.46± 0.29[b]
formed formaldehyde/P-450 (nmoles formed/nmoles P-450/min)	4.14± 0.66	4.76± 0.82	7.13± 0.80[b]	5.74± 0.53
Spectral change				
aniline-cytochrome P-450 ($E_{430-480}$/mg protein) × 10^3	13.6 ± 0.5	16.0 ± 0.7	30.3 ± 1.4[a]	14.1 ± 0.9
δ-ALA synthetase (nmoles/g liver/hr)	27.3 ± 1.6	33.6 ± 2.4	47.5 ± 2.1[a]	34.8 ± 2.7

Rats were pretreated orally with 1,2,3-, 1,2,4- and 1,3,5-TRCB in a dose of 250 mg/kg once daily for 3 days, respectively, and were sacrificed 24 hr after the last administration. All values are mean ± S.E. of 6 rats except phospholipids value is mean ± S.E. of 3 groups of 2 rats.

a) significantly different from control, $p<0.01$

b) significantly different from control, $p<0.05$

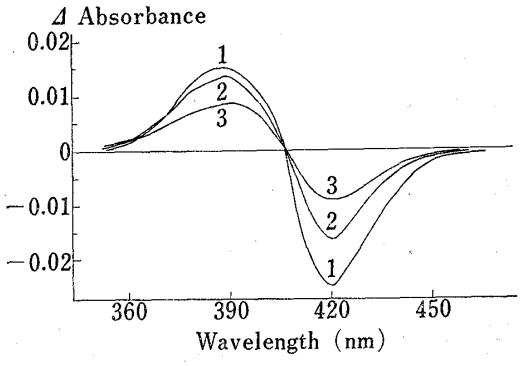

Fig. 2. Spectral Change of Trichlorobenzene-Isomers with Rat Liver Microsomes

Conditions are described in Fig. 1. 1: 1,2,3-TRCB, K_S=0.16 mM; 2: 1,2,4-TRCB, K_S=0.40 mM; 3: 1, 3,5-TRCB, K_S=0.94 mM

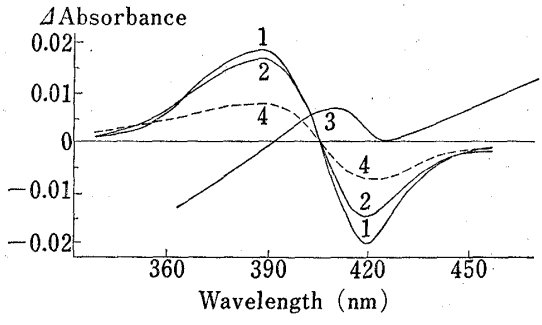

Fig. 3. Spectral Change of Tetrachlorobenzene-Isoners with Rat Liver Microsomes

Conditions except concentration of substrates are described in Fig. 1. Concentrations of substrates of 1,2 and 3 were 1 mM, but 4 was 0.1 mM. 1: 1, 2,3,4-TECB, K_S=0.28 mM; 2: 1,2,3,5-TECB, K_S= 0.46 mM; 3 and 4: 1,2,4,5-TECB, K_S=0.96 mM

Effects of TRCB-isomers

As shown in Table II, the increase of body weight showed the tendency to be depressed by the treatment with 1,2,4-TRCB. The liver weight, contents of glycogen and triglyceride were not affected by the treatment with all isomers except that the liver weight was increased markedly by treatment with 1,2,4-TRCB. However, the contents of both microsomal Pi and protein were increased by the treatment with each isomer. Cytochrome P-450 content was increased approximately 130% by the treatment with 1,2,4-TRCB, in contrast, only 20% and 11% by the treatment with 1,2,3-TRCB and 1,3,5-TRCB, respectively. In addition, cytochrome b_5 content was increased 19%, 13% and 16% by the treatment with 1,2,3-TRCB, 1,2,4-TRCB and 1,3,5-TRCB, respectively. The most intensive inducing effects on drug-metabolizing enzymes were elicited by the treatment with 1,2,4-TRCB, and a similar tendency was noted in both δ-ALA synthetase activity and the magnitude of difference spectrum induced by aniline-cytochrome P-450 binding. The difference spectra induced by each isomer-cytochrome P-450 binding was characterized by type I, as seen in the treatment with DCB-isomers (Fig. 2).

Effects of TECB-isomers

Effects of treatment with TECB-isomers were shown in Table III. The liver weight showed the tendency to be increased by treatment with each isomer. No significant change in triglyceride content was noted, but glycogen content was decreased by the treatment with 1,2,3,4-TECB and 1,2,3,5-TECB. On the other hand, contents of both microsomal Pi and protein were increased by the treatment with each isomer, especially by 1,2,3,5-TECB treat-

Table III. Effects of Treatment with Tetrachlorobenzene Isomers for 3 Days on Hepatic Constituents, Cytochromes, Drug-Metabolizing Enzymes and δ-Aminolevulinic Acid Synthetase

	Control	1,2,3,4-TECB	1,2,3,5-TECB	1,2,4,5-TECB
Body weight initial (g)	119±3	120±3	123±2	119±3
final (g)	131±3	128±3	132±2	129±2
Liver weight (g/100 g b.w.)	5.11± 0.05	5.82± 0.15[a]	5.46± 0 22	5.88± 0.23[a]
Glycogen (mg/g liver)	69.1 ± 2.3	56.3 ± 4.1	42.9 ± 3.2[b]	65.5 ± 4.1
Triglyceride (mg/g liver)	5.2 ± 1.0	4.1 ± 0.4	6.5 ± 0.8	3.9 ± 0.3
Microsomal phosphorus (μg/g liver)	213.0 ±14.5	263.1 ± 7.2	407.4 ± 8.3[b]	286.2 ±25.6
protein (mg/g liver)	16.1 ± 0.3	19.8 ± 0.4[b]	22.2 ± 0.4[b]	20.0 ± 0.5[b]
Cytochromes				
P-450 (nmoles/mg protein)	0.72± 0.02	0.97± 0.05[b]	1.14± 0.03[b]	0.93± 0.02[b]
b_5 (nmoles/mg protein)	0.40± 0.02	0.44± 0.02	0.41± 0.02	0.42± 0.02
Aniline hydroxylase				
formed *p*-aminophenol (nmoles/mg protein/min)	0.66± 0.05	0.74± 0.02	0.76± 0.04	0.70± 0.03
formed *p*-aminophenol/P-450 (nmoles formed/nmoles P-450/min)	0.92± 0.06	0.77± 0.05	0.67± 0.05[a]	0.75± 0.04
Aminopyrine demethylase				
formed formaldehyde (nmoles/mg protein/min)	3.45± 0.36	5.20± 0.38[a]	6.34± 0.79[a]	5.41± 0.23[a]
formed formaldehyde/P-450 (nmoles formed/nmoles P-450/min)	4.80± 0.45	5.38± 0.38	5.56± 0.76	5.82± 0.20
Spectral change aniline-cytochrome P-450 ($E_{430-480}$/mg protein) $\times 10^3$	14.9 ± 0.6	20.3 ± 0.5[b]	21.1 ± 0.2[b]	20.3 ± 0.7[b]
δ-ALA synthetase (nmoles/g liver/hr)	23.8 ± 2.9	33.3 ± 1.6[a]	35.4 ± 3.9[a]	27.5 ± 1.7

Rats were pretreated orally with 1,2,3,4-, 1,2,3,5- and 1,2,4,5-TECB in a dose of 250 mg/kg once daily for 3 days, respectively, and were sacrificed 24 hr after the last administration. All values are mean ± S.E. of 6 rats except phospholipids value is mean ± S.E. of 3 groups of 2 rats.

a) significantly different from control, $p<0.05$

b) significantly different from control, $p<0.01$

ment. Cytochrome P-450 content was increased by the treatment with each isomer, accompanied by enhancement in activity of aminopyrine demethylase, but no significant difference was noted in activity of aniline hydroxylase. In addition, cytochrome b_5 content was not altered. When the enzyme activities were expressed as units of cytochrome P-450, significant decrease was noted in activity of aniline hydroxylase by the treatment with 1,2,3,5-TECB. δ-ALA synthetase activity was enhanced by the treatment with 1,2,3,4-TECB and 1,2,3,5-TECB.

The magnitude of difference spectra induced by aniline-cytochrome P-450 binding was increased significantly. The difference spectra induced by 1,2,4,5-TECB-cytochrome P-450 binding was characterized by type I in low concentration of 1,2,4,5-TECB, 0.1 mM, but the change of the type was noted in high concentration of 1.0 mM (Fig. 3).

Discussion

The presence of optimal molecular sizes or coplanar structure have been confirmed for producing enzyme induction from the studies on polycyclic hydrocarbons,[16] moreover, the studies on DDT derivatives have shown that the most intensive enzyme induction could be caused by the compounds possessing one halogen atom in benzene rings and three halogen atoms in α-position of ethane bridge.[17]

In the present experiments, if the contents of microsomal protein and cytochrome P-450 and the activity of aminopyrine demethylase would be used as indices for measuring biological activities of the chlorinated benzene isomers, *m*-DCB, 1,2,4-TRCB, and 1,2,3,5-TECB could have the strong activities as compared to the other DCB-, TRCB- and TECB-isomers, respectively. From the above, it was suggested that the activities would be related structurally to *meta*-isomers in which last chlorine atom locates each other at the *meta*-position (1,-3; 1,2,-4; 1,2,3,-5) of the benzene ring. However, there would be no obvious explanation, when this was considered only from the view of chlorine-position, for such opposite results that 1,3,5-TRCB had rather weak biological activity. It would probably be considered that these phenomena were in part involved by another factors, the absorption of gastrointestinal tract, tissue concentration, biotransformation, and so on. In general, halogen-substituted benzenes are metabolized in the normal body to phenols, catechols and mercapturic acids, and that the output of each of these metabolites depends on the number and orientation of the halogen atoms. In TRCB-isomers, Jondorf, *et al.*[18] reported that 1,2,3- and 1,2,4-TRCB were metabolized to phenols, 78% and 42%, respectively, but 1,3,5-TRCB was apparently slowly absorbed and metabolized to 2,4,6-trichlorophenol (9%). Similarly 1,2,3,4-TECB was metabolized mainly to phenol (43%), but its isomers, 1,2,3,5- and 1,2,4,5-TECB were oxidized to phenols to only small degree, 5% and 2%, respectively.[19]

Furthermore, each difference spectrum induced by each isomer-cytochrome P-450 binding was characterized by type I. However, large spectral changes were induced specifically by the compounds possessing chlorine atoms at the *ortho*-position each other, that is, *o*(1,2)-DCB, 1,2,3-TRCB and 1,2,3,4-TECB. Moreover, spectral dissociation constant *K*s values of *o*(1,2)-DCB, *m*(1,3)-DCB and *p*(1,4)-DCB were 0.25, 0.56 and 0.77 mM, respectively. Furthermore, the *K*s values of 1,2,3-, 1,2,4- and 1,3,5-TRCB were 0.16, 0.40 and 0.94 mM, respectively, and also 1,2,3,4-, 1,2,3,5- and 1,2,4,5-TECB were 0.28, 0.46 and 0.96 mM, respectively. From these facts, it was possibly suggested that the *o*(1,2)-DCB, 1,2,3-TRCB and 1,2,3,4-TECB would have a marked affinity to microsomal membranes or cytochrome P-450. However,

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the inducing effect of the *ortho*-isomers as mentioned above was rather small as compared to those of the corresponding *meta*-isomers, *i.e.*, *m*(1,3)-DCB, 1,2,-4-TRCB and 1,2,3-5-TECB. These may be exist the chemical structures which were related to binding ability or permeability to the membranes, in addition to the lipophillic properties of the chemical substances themselves.

The enhancement of drug-metabolizing enzyme activities by treatment with each isomers was not always parallel to the increase of cytochrome P-450 content. According to Schulze and Staudinger,[20] there is a close correlation between the changes in contents of phospholipid and cytochrome P-450, and cytochrome P-450 was suggested to be a lipoprotein. Furthermore, the important roles of phospholipid in the mixed-function oxidase system of hepatic microsomes have been demonstrated in several studies.[21] From the findings presented here, the changes in the content of phospholipid under present experiments may cause changes in the enzyme stability or activity of microsomes.

By the treatment with 1,2,4-TRCB and 1,2,3,5-TECB, cytochrome P-450 content was increased 130 and 58%, respectively, while, on the contrary, cytochrome b_5 content was altered as little as 12 and 2%, respectively. It is interesting to consider that this may be due to the difference either in a turn-over rate in both cytochromes or in porphyrine or heme utilization. On the other hand, in the treatment with various isomers δ-ALA synthetase, a rate-limiting enzyme in porphyrine biosynthetic pathway,[22] showed the different activities, but in the treatment with 1,2,4-TRCB there appears to be some relations between moderate increase in δ-ALA synthetase activity and large increase in cytochrome P-450 content as shown in Table II. This finding agreed with that of Baron and Tephly[23] who found that the changes in the cytochrome P-450 content was paralleled, in part, with the changes in the δ-ALA synthetase activity by treatment with phenobarbital or 3,4-benzopyrene. In the treatment with these chlorinated benzenes, the content of hepatic microsomal protein and the liver weight were increased, therefore, the apoprotein of cytochrome P-450 would also increase as a result of stimulation in protein biosynthesis *in vivo*. It may be explained that these increases have caused the intense utilization of heme followed by the enhancement of δ-ALA synthetase activity. Further investigations will be needed for the relations between chemical structures and biological activities of these compounds.

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