Arch. Pharm. Res. 13(3), 265-268

Effects of Lignans on Hepatic Drug-Metabolizing Enzymes

Kuk Hyun Shin, Won Sick Woo, Jung Yun Lee* and Yong Bong Han*

*Natural Products Research Institute, Seoul National University, Seoul 110-460 and *Department of Home Economics, Korea University, Seoul 136-072, Korea*

(Received July 6, 1990)

Abstract□The effects of lignans, related to macelignan, on hepatic microsomal drug-metabolizing enzyme (DME) activity were evaluated to elucidate the structure-activity relationship in mice and rats. The compounds carrying the methylenedioxyphenyl nucleus were found to be the most potent among compounds tested; which not only produced a marked inhibition of DME with a single dose but a significant induction with repeated treatments. Lack of the methylenedioxy group caused marked decrease in the activity, implying that a methylenedioxy group is essential and of major importance eliciting DME modifying activity.

Keywords□Lignans, drug-metabolizing enzymes, enzyme-inhibition, enzyme-induction, cytochrome P-450.

Lignans are group of natural products distributed widely in the plant kingdom, whose carbon skeletons are constructed by the linking of C_6C_3 units, which are formed biogenetially through the shikimate pathway. Biological activities of lignans were found to cover a broad range[1,2] and include anti-tumor, anti-mitotic, anti-viral and anti-microbial properties. A distinct physiological effect was observed with lignan treatment in animals, such as activity on the central nervous system, protective activity against hepatotoxins and general stress reducing activity. On the other hand, lignans were reported to inhibit specifically certain enzymes, such as enzymes affecting respiration, c-AMP phosphodiesterase and catechol-O-methyl transferase.

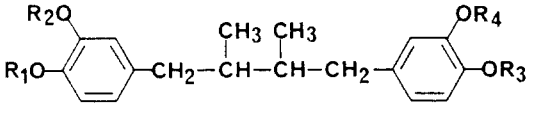
Recently, macelignan, a 1,4-diarylbutane derivative isolated from the arils of *Myristica fragrans* was found to elicit a significant interaction with drug-metabolising enzymes (DME)[4].

This paper aims to investigate to help elucidation of the structure-activity relationship on inhibitory effect of diarylbutane type lignans.

MATERIALS AND METHODS

Chemicals

Macelignan (I) and meso-dihydroguaiaretic acid (II) were isolated from mace[5]. 3-Demethyl-meso-dihydroguaiaretic acid (III), 1-(3,4-methylenedioxyphenyl)-2,3-dimethyl-4-(3'-4'-dimethoxybenzyl) butane (IV), meso-dihydroguaiaretic acid dimethyl ether (VI) and 1,4-bis-(3,4-methylenedioxybenzyl)-dimethylbutane (VII) were synthesized[6]. Nor-dihydroguaiaretic acid (V) was purchased from Sigma Chem. Co. SKF-525A was kindly supplied by Smith, Kline and French Lab.



Compounds	R_1	R_2	R_3	R_4
I	-CH_2-		H	CH_3
II	H	CH_3	H	CH_3
III	H	H	H	CH_3
IV	-CH_2-		CH_3	CH_3
V	H	H	H	H
VI	CH_3	CH_3	CH_3	CH_3
VII	-CH_2-		-CH_2-	

Chart 1

Animals

Male mice weighing 18-25g of dd strain were used for *in vivo* experiments and male Spague-Dawley rats weighing 150-200g of CD strain for *in vitro* enzyme assays.

Part 15 in the series "Studies on crude drugs action on drug-metabolizing enzymes" For part 14, see ref 1.

Bioassays

The effects on hepatic DME were evaluated by measuring the duration of sleeping time induced by hexobarbital, strychnine mortality, aminopyrine N-demethylase activity as well as cytochrome P-450 content in microsomes according to the methods described previously[7,8,16]. In brief, the duration of sleep was measured by injecting hexobarbital sodium (50 mg/kg, i.p.) to mice 30 min after a single treatment or by injecting 100 mg/kg i.p. 48 hr after the last dose of 4 daily pretreatments of compounds. The strychnine mortality was estimated by injecting strychnine nitrate (1.2 mg/kg, i.p.) 30 min after the sample treatment. Mice were observed for 30 min and the mortality was recorded. Aminopyrine N-demethylase activity was measured by using 10,000g supernatant fraction of liver homogenates as enzyme source. Compounds were solubilized in EtOH, the final concentration being 1.96%[16]. The concentration of cytochrome P-450 was measured from the difference in absorbance of the reduced CO-cytochrome P-450 complex between 450 and 490 nm[7].

RESULTS AND DISCUSSION

The effect of a single treatment with lignans on hexobarbital-induced sleeping time, strychnine mortality and aminopyrine-N-demethylase activity were estimated in mice and the results were shown in Table I. The mean duration of hexobarbital-induced sleep of the animal group treated with SKF-525A, a prototype enzyme inhibitor, at a dose of 30 mg/kg, i.p. was markedly prolonged by about 6.6 times compared to that of the control. Although far less active than this compound, it was shown that, among substances tested, compound IV and VII exhibited the considerable prolongation of the sleep duration at a dose of 200 mg/kg, by about 5.5 times compared to that of the control. At the same dosage level, compounds III, V and VI showed little or no potentiation of the sleeping time. Compounds I and II showed a moderately significant prolongation (167.2 and 82.6% increase, respectively). The pattern of synergism induced by strychnine was quite similar to those for the potentiation of the barbiturate-induced sleeping time which appeared to closely reflect their effects of the microsomal enzymes rather than a simple potentiation due to their CNS depressant action. The strychnine-induced mortality was markedly enhanced in animal groups treated with compounds IV and VII. Compounds I and II exhibited potentiation of the toxicity corresponding to those of the barbiturate-induced action. Evidence supporting the liver enzyme inhibitory effect of lignans was demonstrated by measurement of aminopyrine N-demethylase activity. Compounds IV and VII exhibited most potent inhibitory activity (71.2% and 63.3% inhibition, respectively). Compounds I and II were significant but less active, whereas compounds III, V and VI showed no significant inhibitory activity at the same dosage level. The direct effect of the compounds on hepatic DME was evaluated by estimating the ability of the compounds

Table I. Effects of lignans on hexobarbital induced hypnosis, strychnine mortality and aminopyrine N-demethylase activity in mice

Treatment	Dose (mg/kg, i.p.)	Sleeping time[a] (min)	Strychnine mortality (No. died/No. dosed)	Aminopyrine N-demethylase[b] (nmoles/30 min/mg prot.)
Control	–	24.7± 1.7	2/10	17.84±2.39
SKF-525A	30	162.8±10.2***	10/10	8.41±0.48**
Compound I	200	66.0± 2.3**	9/10	11.77±0.29*
II	200	45.1± 3.5*	4/10	13.73±0.72*
III	200	29.3± 5.4	2/10	16.90±2.40
IV	200	132.2±11.1***	10/10	5.14±1.70**
V	200	32.0± 3.6	3/10	17.25±1.05
VI	200	29.0± 3.2	3/10	19.85±1.44
VII	200	134.7± 7.2***	10/10	6.55±1.60**

Mice were treated with compounds suspended in 0.5% CMC 30 min prior to bioassays.

[a] Each value is the mean±S.E. for five to six animals.

[b] Each value is the mean±S.E. for triplicate determinations.

Significantly different from the control; *$p<0.05$, **$p<0.01$, ***$p<0.001$.

to inhibit aminopyrine N-demethylase activity *in vitro*. Table II shows the inhibitory potencies expressed as IC_{50} values which were derived from regression equations obtained by plotting relative velocity vs. the inhibitory concentrations. The most potent compounds in the series were those carrying a piperonyl group (compounds I, IV and VII). The IC_{50} values of which were 75μM, 44μM and 59μM, respectively. Compounds which are devoid of a piperonyl group elicited considerable decrease or complete loss in the inhibitory activity. The present investigation has clearly demonstrated that a methylenedioxy group in aromatic ring may play a significant role in eliciting DME inhibitory activity. It was reported that sesamin and asarinin were effective in enhancing the toxicity of a wide variety of insecticides[9,10]. However, the related compound, pinoresinol and its dimethyl ether are inactive. These observations point towards the importance of the methylenedioxy substituent in synergistic activity. Other studies have shown clearly that the methylendioxy group is responsible for the inhibition of mixed function oxidases[11-13], the enzyme system that is responsible for the oxidation and inactivation of most toxins. It is possible that these compounds share this common biological activity as a result of the presence of the methylenedioxy group. However, meso-dihydroguaiaretic acid (compound II) which is devoid of a methylenedioxy group showed a distinct effect even if its intensity is weak. Thus, the chemistry underlying this biological phenomenon may not be quite so straightforward. The fact that its dimethylether (compound V) showed no activity suggests that such phenomena may apparently result from such factors as lipoid solubility, steric hindrance and/or binding on the active surface of the target enzymes.

Table II. Inhibition of aminopyrine N-demethylase by lignans *in vitro*

Inhibitors	IC_{50}* (μM)
SKF-525A	10
Compound I	75
II	92
III	–
IV	44
V	–
VI	–
VII	59

Microsomal fraction (10,000g supernatant fraction) from livers of untreated rats were assayed for N-demethylase activity using a fixed concentration of aminopyrine (0.82 mM) as substrate.
Assay was run with graded concentrations of the inhibitors.
*Concentration of an inhibitor required to reduce the rate of N-demethylation by 50%.

Table III. Effects of repeated treatments of lignans on hexobarbital-induced hypnosis, aminopyrine N-demethylase and cytochrome P-450 in mice

Treatment	Dose (mg/kg, i.p.)	Sleeping time[a] (min)	Aminopyrine N-demethylase[b] (nmoles/30 min/mg prot.)	Cytochrome P-450[b] (nmoles/mg prot.)
Control	–	43.0 ± 1.1	23.81 ± 2.72	0.87 ± 0.09
Phenobarbital	50	14.8 ± 2.2***	47.23 ± 2.19**	1.92 ± 0.06***
Compound I	75	25.4 ± 1.4*	38.83 ± 3.25*	1.47 ± 0.22
II	75	39.4 ± 2.7	34.79 ± 2.06*	1.26 ± 0.08
III	75	44.6 ± 1.4	34.32 ± 3.23	0.83 ± 0.16
IV	75	20.9 ± 1.5*	56.98 ± 1.50***	1.87 ± 0.09***
V	75	53.4 ± 6.1	23.90 ± 2.82	1.09 ± 0.02
VI	75	54.7 ± 9.3	24.36 ± 2.41	0.77 ± 0.03
VII	75	23.4 ± 0.9*	63.03 ± 2.64***	1.99 ± 0.24***

Mice were pretreated with compounds daily for 4 days and sleeping time and enzyme activities were measured 48 hrs after the last dose.
[a] Each value is the mean ± S.E. for 5 to 6 animals.
[b] Each value is the mean ± for the triplicate determinations.
Significantly different from the control; *$p<0.05$, **$p<0.01$, ***$p<0.001$.

Drugs that initially inhibit the activity of the microsomal DME when given acutely, often stimulate the biotransformation of other drugs when administered chronically[14]. SKF-525A produced such an effect[15] and even in our earlier experiments, naturally occurring furanocoumarins which have been demonstrated as strong inhibitors, significantly induced hepatic DME with repeated treatments of them[16].

In this experiment, distinct biphasic responses on microsomal enzymes was also demonstrated. As shown in Table III, compounds I, II, IV and VII, with 4 daily consecutive administrations (75 mg/kg, i.p.) in mice, not only produced significant shortening of the barbiturate-induced sleep duration but caused significant enhancements of aminopyrine N-demethylase activity. Increases in microsomal cytochrome P-450 contents were also seen although those of the animals treated with compound I and II did not reach the level of statistical significance. The extent of the enzyme induction produced by compounds IV and VII were quite remarkable, their activities being approximately comparable to those of the animal group treated with phenobarbital (50 mg/kg, i.p.), which is known as a typical enzyme inducer[17].

ACKNOWLEDGEMENT

This work was supported in part by a research grant from Korea Science and Engineering Foundation.

LITERATURE CITED

1. Shin, K.H. and Woo, W.S.: Inhibition and induction of hepatic mixed function oxidase by phenylpropanoids from the seeds of *Myristica fragrans, Kor. Biochem. J.*, **23**, 122 (1990).
2. Macrae, W.D. and Towers, G.H.N.: Biological activities of lignans, *Phytochem.* **23**, 1207 (1984).
3. Whiting, D.A.: Lignans, neolignans and related compounds. *Natural Product Rep.* **4**, 499 (1987).
4. Shin, K.H. and Woo, W.S.: Biological evaluation of mace for drug metabolism modifying activity, *Kor. J. Pharmacogn.* **17**, 189 (1986).
5. Woo, W.S., Shin, K.H., Wagner, H. and Lotter, H.: The structure of macelignan from *Myristica fragrans, Phytochem.* **26**, 1542 (1987).
6. Lee, J.Y., Han, Y.B., Woo, W.S. and Shin, K.H.: Antioxidant activity of lignans related to NDGA, *Kor. J. Pharmacogn.* in press (1990).
7. Shin, K.H. and Woo, W.S.: Inhibition of hepatic microsomal drug metabolizing enzymes by imperatorin, *Arch. Pharm. Res.* **9**, 81 (1986).
8. Shin, K.H. and Woo, W.S.: Effects of psoralen and angelicin on hepatic drug-metabolizing enzyme activities, *Arch. Pharm. Res.* **11**, 122 (1988).
9. Haller, H.L., McGovran, E.R., Goodhue, L.D. and Sullivan, W.N.: The synergistic action of sesamin with pyrethrum insecticides, *J. Org. Chem.* **7**, 183 (1942).
10. Haller, H.L., LaForge, F.B. and Sullivan, W.N.: Some compounds related to sesamin: Their structures and their synergistic effect with pyrethrum insecticides, *J. Org. Chem.* **7**, 185 (1942).
11. Casida, J.E.: Mixed-function oxidase involvement in the biochemistry of insecticide synergists, *J. Agric. Food Chem.* **18**, 753 (1970).
12. Graham, P.S., Heller, R.O. and Ryan, A.J.: The inhibition of drug metabolism enzymes by some naturally occurring compounds, *Biochem. Pharmacol.* **19**, 759 (1970).
13. Graham, P.S., Heller, R.O. and Ryan, A.J.: The Kinetic of inhibition of drug metabolism *in vitro* by some naturally occurring compounds, *Biochem. Pharmacol.* **19**, 769 (1970).
14. Peterson, G.R., Hostetler, R.M., Lehman, T. and Covault, H.P.: Acute inhibition of oxidative drug metabolism by propoxyphene, *Biochem. Pharmacol.* **28**, 1783 (1979).
15. Anders, M.W. and Mannerings, G.J.: Inhibition of drug metabolism. I. Kinetics of the inhibition of the N-demethylation of ethylmorphine by 2-diethylaminoethyl-2, 2' diphenylvalerate HCl and related compounds, *Molec. Pharmacol.* **2**, 319 (1966).
16. Woo, W.S., Shin, K.H. and Lee, C.K.: Effect of naturally occurring coumarins on the activity of drug metabolizing enzymes, *Biochem. Pharmac.* **32**, 1800 (1983).
17. Conney, A.H., Davidson, C., Gastel, R. and Burns, J.J.: Adaptive increases in drug-metabolizing enzymes induced by phenobarbital and other drugs, *J. Pharmacol. Exp. Ther.* **130**, 1 (1960).