생 약 학 회 지
Kor. J. Pharmacogn.
17(1) : 91~99 (1986)

Hepatic Drug Metabolism Modifier from Arils of *Myristica fragrans*

Kuk Hyun Shin and Won Sick Woo

Natural Products Research Institute, Seoul National University, Seoul 110, Korea

Abstract—The single treatment of mice with steam distillate, non-volatile ether extract and methanol extract from mace (Arils of *Myristica fragrans*) caused a significant prolongation of hexobarbital-induced narcosis and increase in strychnine toxicity as well as a significant decrease in hepatic microsomal drug metabolizing enzyme activities. On 7 consecutive daily administrations, however, the duration of hypnosis was markedly shortened and significant increases in the hepatic enzyme activities were shown. With systematic fractionation by SiO_2 column chromatography of non-volatile ether fraction monitoring by animal tests a new lignan (mp 70~72°, MW 328, $[\alpha]_D^{20}+5.28$) was isolated as an active principle and its structure was elucidated as (2R, 3S)-1-(3, 4-methylendioxyphenyl)-2, 3-dimethyl-4-(4-hydroxy-3-methoxyphenyl) butane.

Keywords—*Myristica fragrans* · Myristicaceae · mace lignan · drug metabolizing enzyme activities

Various environmental factors such as drugs, pesticides, carcinogens and food additives have been demonstrated to influence hapatic oxidative metabolism of other drugs and chemicals in man and animals, changing markedly the intensity of therapeutic or toxic activity of many drugs.[1]

In the course of characterization of such hepatic drug metabolism modifiers from plant resources, we have performed successive screening on crude extracts of 150 widely employed medicinal plants[2] as well as 22 spices,[3] utilizing animal experimental models of hexobarbital (HB)-induced sleeping time and strychnine (ST) mortality test. As a result, we have found that at least 30% of plant materials tested affected hepatic drug metabolizing function. On the basis of these screening results, we have been pursuing active constituents from active plant materials.[4-8]

This report deals with isolation and structure elucidation of an active lignan from mace, the arilode of *Myristica fragrans* (Myristicaceae), a well known spice, the crude extract of which was found to cause a strong modification of hepatic drug metabolizing enzyme (DME) activities.

Effect of Mace Extracts on DME Activities

The ether extract and methanol extract obtained from the marc after ether extraction were initially tested for their effect on hepatic DME function in mice. Table I shows the results of the effect of these extracts on the duration of HB-induced sleeping time and ST-mortality in mice. SKF-525A,[9] a well known enzyme inhibitor and phenobarbital,[10] a well known enzyme inducer were used as reference compounds. Both extracts showed a significant prolongation of sleeping time at a dose of 200 mg/kg ip in

Table I. Effects of Et_2O ex. and MeOH ex. of mace on hexobarbital induced hypnosis and strychnine mortality in mice

Treatment	Hexobarbital hypnosis						Strychnine mortality[c] (No. died/ No. used)
	Phase I [a]			Phase II [b]			
	Number of mice	Mean±S.E. (min.)	Percent of control	Number of mice	Mean±S.E. (min.)	Percent of control	
Control (0.5% CMC)	6	23.5±1.6	—	6	65.2±5.1	—	3/10
SKF-525A (30 mg/kg)	6	178.4±14.3***	759.3	6	—	—	10/10
Phenobarbital-Na (50 mg/kg)	6	—	—	6	22.3±2.2***	34.4	—
Et_2O Ex. (200 mg/kg)	6	50.6±5.4***	215.5	6	37.1±2.1***	57.1	9/10
MeOH Ex. (200 mg/kg)	6	42.9±3.2***	182.6	6	41.5±3.0**	63.7	7/10

a) A single treatment 30min before test.
b) Seven consecutive daily treatments 48hr before test.
c) Strychnine nitrate; 1.10 mg/kg, i.p.
Significantly different from the control; **$p<0.01$, ***$p<0.001$

Table II. Effect of an acute treatment with mace extracts on DME activities in mice

	Treatment			
	Control (0.5% CMC)	SKF-525A (30 mg/kg, i.p.)	Ether Ex. (200 mg/kg, i.p.)	MeOH Ex. (200 mg/kg, i.p.)
Aminopyrine N-demethylase (μ moles/g·prot./30 min)	15.6±0.7	6.4±0.6** (59.0)	7.7±1.5** (50.6)	7.6±1.3** (51.3)
Hexobarbital hydroxylase (μ moles/g·prot./hr)	26.4±1.5	12.9±1.5** (51.1)	16.6±1.7* (37.1)	17.7±4.6* (33.0)
Aniline hydroxylase (μ moles/g·prot./30 min)	5.3±0.2	—	2.0±0.2** (62.3)	2.7±0.2* (49.1)

Animals were killed 30 min after a single treatment.
Each data represents mean±S.E. of 3 separate determinations.
Figures in parentheses indicate % inhibition.
Significantly different from the control; **$p<0.01$, *<0.05.

the first phase experiments, namely when tested 30 min after a single treatment which was 1/4 as potent as 30 mg/kg of SKF-525A.

This prolongation of sleeping time was not considered to be due to a CNS-depressant action but due to inhibition of hepatic drug metabolism because the treatment of the extracts also increased toxicity of strychnine which is mainly metabolized enzymatically in hepatic DME system.[11] On the other hand, these extracts significantly shortened the duration of sleeping time in the second phase experiments, namely when tested 48hr after the last of 7 daily consecutive treatments.

From these results, it was presumed that the mace extracts had a biphasic response on hepatic DME system, both inhibitory and inducing effect. In order to confirm this assumption, direct effects of the mace extracts on the metabolism of some substrates such as aminopyrine, hexobarbital and aniline in both phases were evaluated and the results were indicated in Table II.

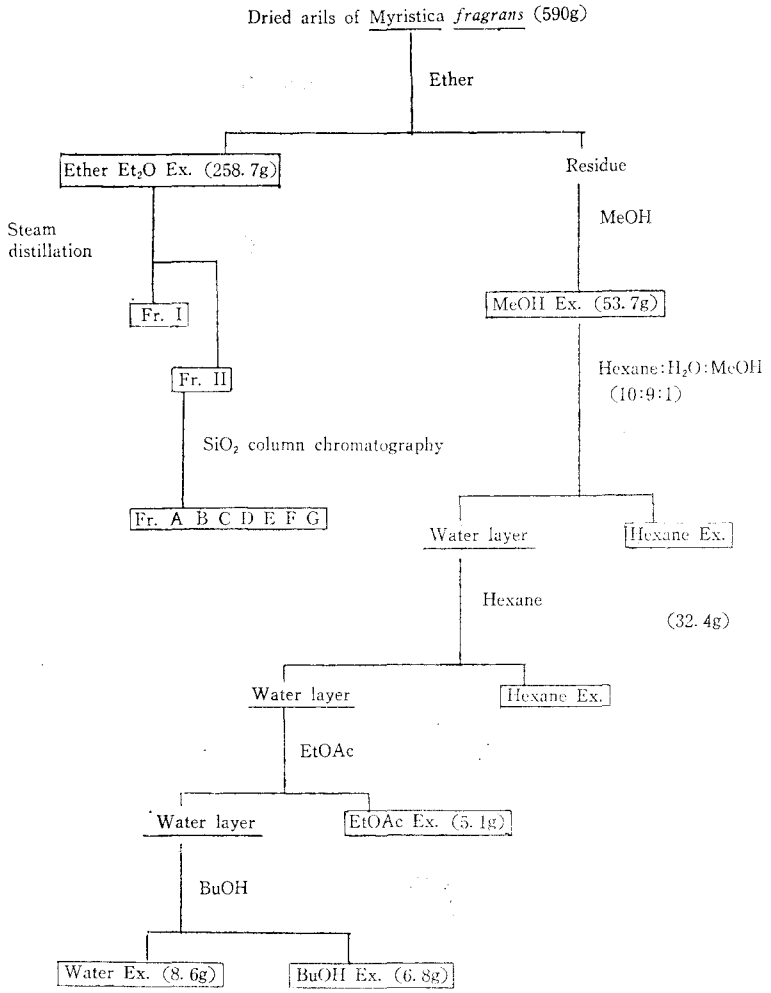
Coincidence with the barbiturate induced sleeping time, the enzyme activities of livers 30min after a single treatment of both extracts were significantly reduced; about 50% in aminopyrine N-demethylase; 37 or 33% in hexobarbital hydroxylase; 62 or 49% in aniline hydroxylase compared to those of the control.

As shown in Table III, on repeated treat-

Table III. Effect of repeated treatments with mace extracts on DME activities and cytochrome p-450 content in mice

	Treatment[a)]			
	Control (0.5% CMC)	Phenobarbital (50 mg/kg/day, p.o.)	Ether ex. (200 mg/kg/day, p.o.)	MeOH ex. (200 mg/kg/day, p.o.)
Aminopyrine N-demethylase (μmoles/g·prot./30 min)	8.7±0.7	16.7±0.9*** (192.5)	14.6±2.0** (167.8)	13.2±1.1** (151.7)
Hexobarbital hydroxylase (μmoles/g·prot./hr)	21.9±0.7	51.4±2.1*** (234.7)	35.8±3.4** (163.5)	34.5±5.6* (157.5)
Aniline hydroxylase (μmoles/g·prot./30 min)	4.1±0.2	6.2±0.3** (150.1)	4.0±0.4 (97.6)	4.2±0.1 (101.7)
Cyt. p-450 (μmoles/mg prot.)	0.76±0.06	1.69±0.03*** (222.4)	0.95±0.02* (125.0)	0.92±0.05 (121.0)

a) Seven daily consecutive treatments 48hr before test.
b) Data represent mean±S.E. of 3 separate determinations.
Figures in parentheses indicate % of the control.
Significantly different from the control; ***$p<0.01$, **$p<0.02$, *$p<0.05$.



Scheme 1. Fractionation of mace.

ments with the extracts, however, aminopyrine N-demethylase and hexobarbital hydroxylase activities were significantly elevated by 67.8% in case of the ether extract and 51.7% in case of the methanol extract compared to those of the control, although aniline hydroxylase activity was unaffected which is somewhat different from phenobarbital treatment which caused a significant elevation of aniline hydroxylase activity. The level of cytochrome p-450 which is a terminal enzyme in DME system was also significantly elevated in the case of the treatment of ether extract.

Based on the above results, we attempted a further systematic fractionation of the mace extracts as indicated in Scheme 1. The ether extract was steam distillated dividing into steam distillate (Fraction I) and non-volatile ether fraction (Fraction II). The methanol extract was fractionated into hexane, ethylacetate and butanol fractions and the efficacy of each fraction was compared by barbiturate-induced sleeping time and ST-mortality test and the results were shown in Table IV.

Among various fractions tested, the steam distillate, non-volatile ether fraction and hexane fraction were shown to cause a significant prolongation and potentiation of ST-mortality. From the first fraction obtained by elution with hexane, ether on silica gel column chromatography of non-volatile ether fraction gave a single crystal as an active principle; it was shown that this

Table IV. Effects of various fractions of mace on hexobarbital induced hypnosis and strychnine mortality

Treatment	Hexobarbital hypnosis						Strychnine mortality[c] (No. died/ No. used)
	Phase I [a]			Phase II [b]			
	Number of mice	Mean±S.E. (min.)	Percent of control	Number of mice	Mean±S.E. (min.)	Percent of control	
Control	5	25.0±3.7	—	5	72.2±4.5	—	3/10
Et_2O Fr. I	5	69.7±2.4***	278.7	5	30.8±2.5***	42.7	8/10
Et_2O Fr. II	5	51.4±3.2***	205.6	5	52.0±6.1**	72.0	9/10
Hexane Ex.	5	61.8±2.5***	247.3	5	47.0±4.1**	65.1	6/10
EtOAc Ex.	5	47.4±8.6*	189.6	5	47.8±1.3**	66.2	—
BuOH Ex.	5	24.1±5.3	96.5	5	67.4±3.7	93.4	—
H_2O Ex.	5	40.0±3.0*	160.1	5	57.3±2.7*	79.4	—

a) A single treatment (200 mg/kg, p.o.) 30 min before test.
b) Seven daily consecutive treatments 48hr. before test.
c) Strychnine nitrate; 1.10 mg/kg, i.p.
Significantly different from the control; *$p<0.05$, **$p<0.01$, ***$p<0.001$

Table V. Effect of mace lignan on hexobarbital-induced hypnosis, strychnine mortality and aminopyrine metabolism *in vitro*

Treatment	Hexobarbital hypnosis (min±S.E.)	Strychnine mortality[b] (No. died/No. used)	Aminopyrine N-demethylase[c] (μmoles/30-min/g prot.)
Control	27.5±2.5(5)	3/10	15.4±0.9
Lignan (100 mg/kg, i.p.)	88.8±5.7(5)*** (322.9)[a]	7/10	10.4±0.3*** (67.5)

Significantly different from the control : ***$p<0.001$
a) Percent of the control.
b) Strychnine nitrate : 1.10 mg/kg, i.p.
c) Data represent mean±S.E. of 3 separate determinations.

compound, at a dose of 100 mg/kg ip, caused a significant prolongation of HB-induced sleeping time, increased ST-mortality as well as exhibited a significant inhibitory activity on aminopyrine metabolism *in vitro*. (Table V)

Structure Elucidation of Mace Lignan

The mace lignan ($C_{20}H_{24}O_4$), mp. 70~72° was crystallized as a transparent prism from hexane : ether (1 : 1). It was optically active, its $[\alpha]_D^{20} = +5.28°$ in chloroform. It gave green color with ethanolic $FeCl_3$, but was negative in Gibbs and Emerson test.

The UV spectrum of this lignan in methanol showed three maxima at 213 nm (logε, 3.972), 230nm (logε, 3.88) and 285 nm (logε, 3.769), Positive response in $FeCl_3$ test and a bathochromic shift of UV spectra with the addition of alkaline solution indicated that it is a simple

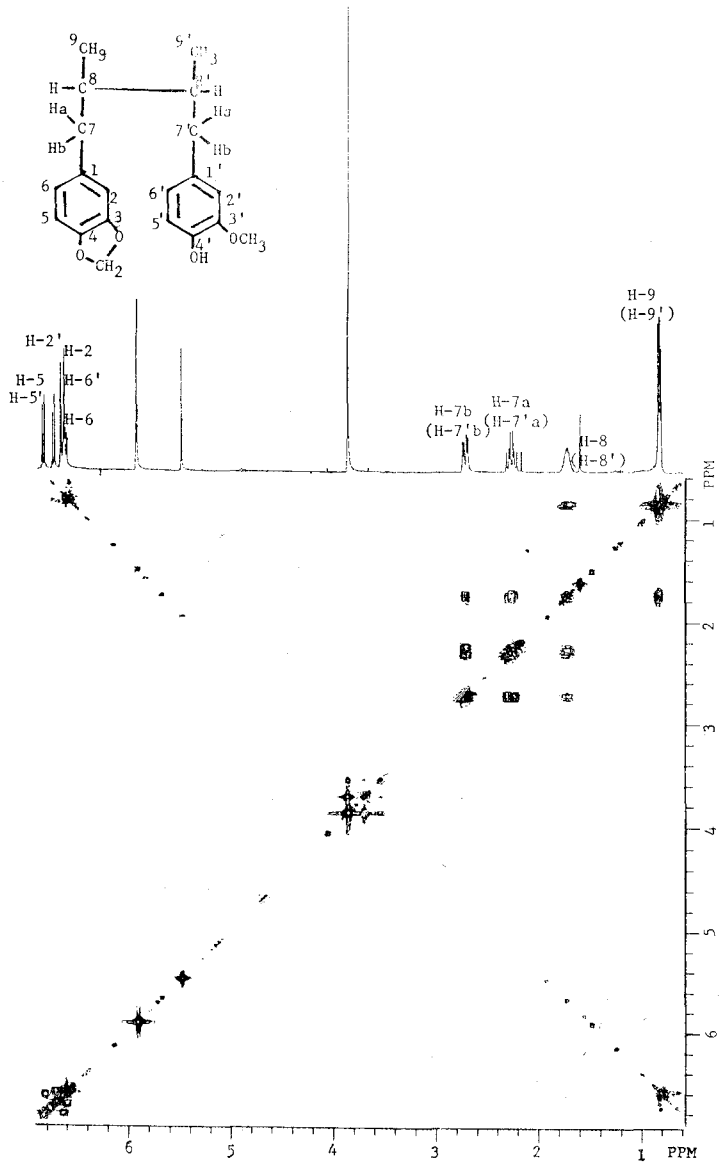


Fig. 1. Proton homonuclear correlation 2D-contour plot display of mace lignan.

aromatic benzenoidal substance with free hydroxyl group, but without any conjugated group.

IR spectra showed absorption bands at 3,480 cm^{-1} region, four strong absorption bands in the range of 1,440 and 1,514 cm^{-1} and three strong bands at 1,265 and 1,232 cm^{-1} indicating the presence of phenolic hydroxyl group.

From two weak combination and overtone absorpotion bands at 1,740 and 1,840 cm^{-1}, and bands at around 800 cm^{-1} and 925 cm^{-1}, it could be postulated that this compound has substitution pattern of typical 1, 2, 4-substituted benzenoidal type compound.

EI-MS displayed M^+ at m/z 328 (Rel. int; 11%) and the base peak at m/z 137 (100%) provided the evidence for the presence of a methoxyl and a hydroxyl group in a benzyl unit, and also a prominent peak at m/z 135 (68%) indicated the presence of methylene-dioxy group substituted benzyl unit.

The H^1-nmr spectrum (80 MHz, $CDCl_3$) revealed a sharp doublet centered at 0.84 ppm (J= 6.6Hz) and integrating for six protons of the two methyl groups; a complex multiplet ranging from 1.4 to 1.8 ppm of the two methine protons; multiplet ranging from 2.2 to 2.8ppm of four methylene protons; a sharp singlet at 3.86 ppm accounting for a methoxy group; a sharp singlet at 5.91 ppm accounting for a methylene-dioxy group; a multipletdue to aromatic protons ranging from 6.59 to 6.81 ppm accounting for two benzene rings. In order to establish the mode of couplings contributing to each multiplet, 2D-nmr was measured at 360MHz. Fig. 1 shows proton homonuclear correlation 2D contour plot display of the lignan. It can be observed that H-9(H-9′) representing two methyl groups coupled with H-8(H-8′) which represent two methine protons,; Two methine protons H-8(H-8′) again coupled with H-7a(H-7′a) or H-7b(H-7′b); H-7a(H-7′a) again coupled with H-7b(H-7′b) protons.

Expansion of ^{1}H-nmr spectrum showed more clearly the mode of couplings between two

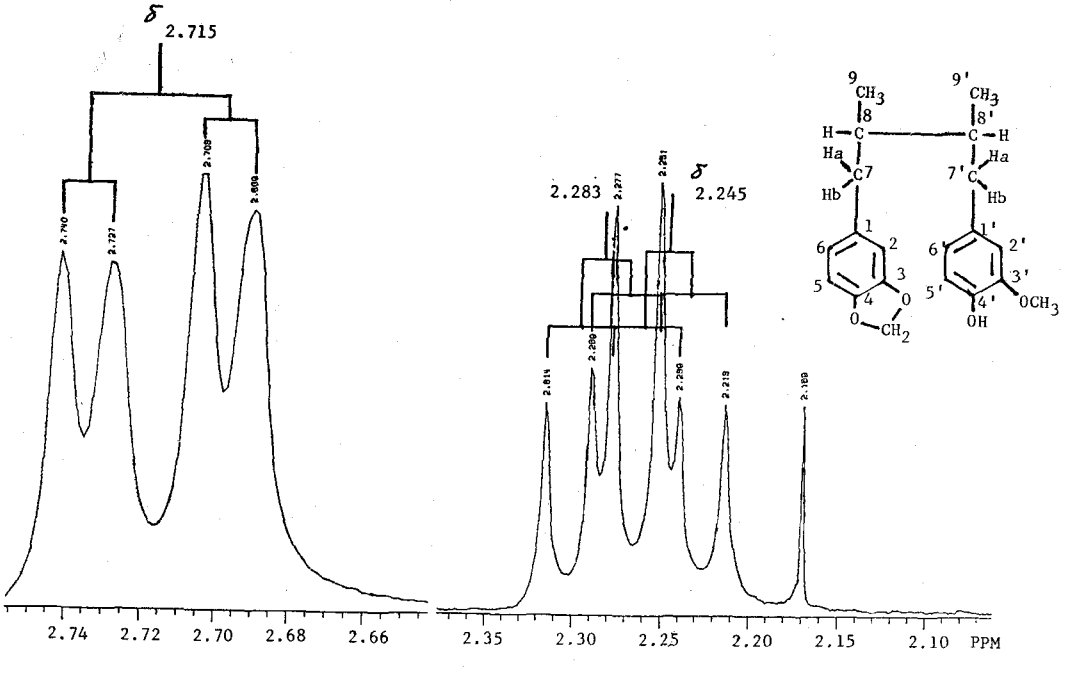


Fig. 2. Expanded ^{1}H-nmr spectrum of mace lignan.

methine protons and 4 benzylic methylene protons as shown in Fig. 2. From 2.2 to 2.33 ppm, six peaks could be observed as a result of overlap of two double doublet due to geminal coupling between H-7a and H-7b (dd, $J_{7a,7b}$=13.68 Hz) and vicinal coupling between H-7a and H-8 (dd, $J_{7a,8}$=9.36 Hz).

A broad doublet in the range from 2.68 to 2.76 ppm could be observed due to geminal couplings between H-7'b and H-7'a ($J_{7'a,7'b}$=13.68 Hz) and vicinal couplings between H-8' and H-7'a (dd, $J_{7'a,8'}$=4.86).

Far small J value between H-8' and H-7'b than between H-7a and H-8 is considered to be due to a difference in the dihedral angle α between these two bonds.

Fig. 3. shows the expanded 2D-nmr spectrum for six aromatic protons which appeared as 12 peaks in the range from 6.58 to 6.84 ppm. Two doublets centered at 6.822 ($J_{6',5'}$=7.92 Hz) and

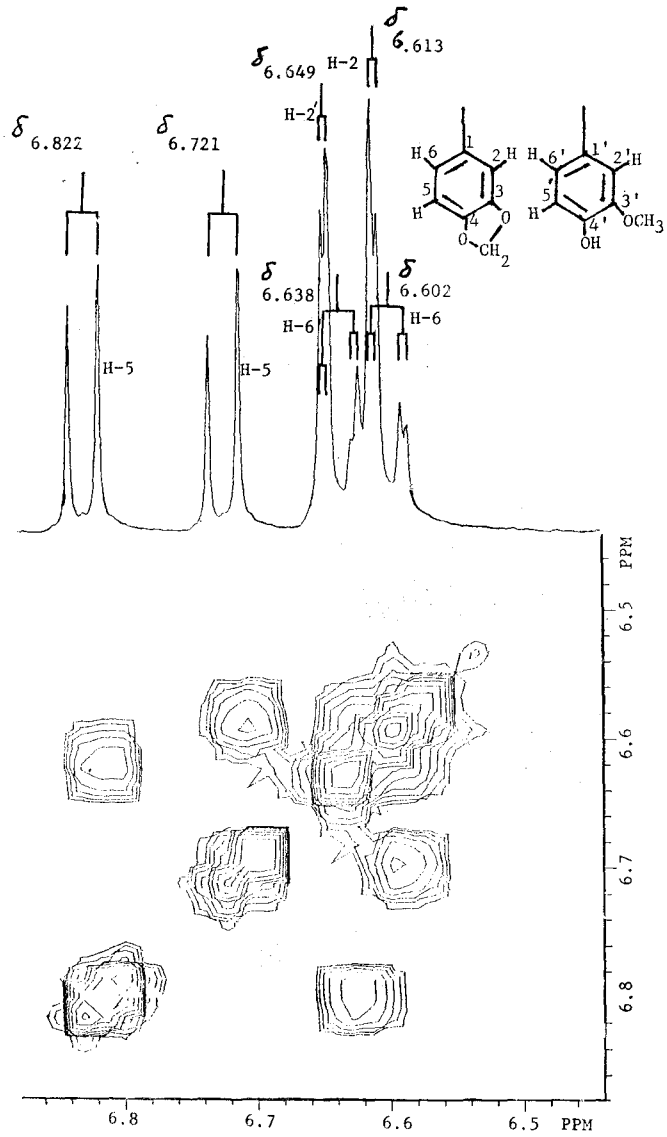


Fig. 3. 2D-contour plot of Mace lignan (expanded)

Table VI. ^{13}C NMR chemical shifts of mace lignan

Carbon No.	Chemical shift (ppm)	Carbon No.	Chemical shift (ppm)
1	133.76	1′	135.73
2	108.00	2′	111.74
3	145.90	3′	147.50
4	143.78	4′	139.80
5	109.43	5′	114.24
6	121.86	6′	121.86
7	38, 99[a)]	7′	39.21[a)]
8	39.39	8′	39.39
9	16.17[b)]	9′	16.25[b)]
—O—CH_2—O—	100.7	—OCH_3	55.92

a, b) Assignment may be reversed.

9.721 ($J_{6,5}$=7.92 Hz) due to ortho couplings of H-6′ with H-5′ and H-6 with H-5 protons, respectively, two doublets centered at 6.649 ($J_{2',6'}$=1.44 Hz) and 6.613 ($J_{2,6}$=1.44 Hz) due to metha couplings of H-6′ with H-2′ and H-6 with H-2, and two double doublets centered at 6.638 and 6.602 (dd, $J_{6',5',2'}$ and dd, $J_{6,5,2}$= 7.92 Hz) due to ortho and metha couplings of H-6′ with H-5′ and H-2′ and H-6 with H-5 and H-2 respectively.

From above results it could be attributable that the functional groups are attached at 3 and 4 position in each benzene ring. And further, negative responses in Gibbs and Emerson tests indicated that hydroxyl group should be at 4 position.

Further confirmation of the structure of this lignan was made by a ^{13}C-nmr spectral analysis and the chemical shifts of the signals corresponding to carbon numbers of this compound were listed in Table Ⅵ. Signals for two methyl carbons at 16.25 and 16.17 ppm; for two methylene carbons at 39.21 and 38.99 ppm; for two methine carbons at 39.39 and 39.39 ppm; for one methoxyl carbon at 55.92 ppm; for one methylenedioxy carbon at 100.7 ppm were appeared, respectively.

Six aromatic tertiary carbons in the range from 107 to 122 ppm and six quaternary aromatic carbons in the range from 133 to 148 ppm could also be observed.

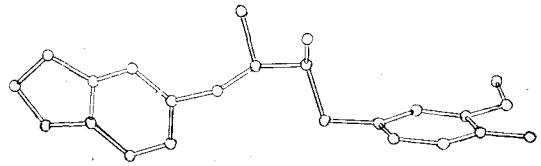


Fig. 4. Absolute configuration of mace lignan by X-Ray crystallography.

In order to elucidate stereochemical configuration, the chemical conversions on this compound were carried out as followings; After selective cleavage of the methylenedioxy group with borontrichloride in methylenechloride,[12] followed by methylation with dimethylsulfate and potassium carbonate in the usual manner a methyl ether derivative, mp 96~98°C and optically inactive compound was obtained. Methylation of nordihydroguaiaretic acid which has been characterized to be cis configuration gave the same methylether derivative. Hence, this lignan was found to be cis orientation.

For the complete stereochemical study, this compound was subject to the X-ray crystallographic analysis. Fig. 4. shows an ORTEP drawing deduced from X-ray analysis. The structure of this lignan was, therefore, established as (2R, 3S)-1-(3, 4-methylendioxyphenyl)-2, 3-dimethyl-4 (4-hydroxy-3-methoxylphenyl)-butane.

Acknowledgment—This work was supported in part by a research grant from KOSEF.

Literature Cited

1. Conney, A.H.: *Pharmacol. Rev.* **19**, 317 (1967).
2. Shin, K.H. and Woo, W.S.: *Kor.J. Pharmacog.* **11**, 109 (1980).
3. Han, Y.B., Shin, K.H. and Woo, W.S.: *Arch. Pharm. Res.* **7**, 53 (1984).
4. Woo, W.S., Lee, C.K. and Shin, K.H.: *Planta Med.* **45**, 234 (1982).

5. Woo, W.S., Shin, K.H. and Lee, C.K.:*Biochem. Pharmacol.* **32**, 1800 (1983).
6. Woo, W.S., Shin, K.H. and Kim, Y.S.: *Ann. Rep. Nat. Prod. Res. Inst.* **18**, 12 (1979).
7. Shin, K.H., Yun, H.S., Woo, W.S. and Lee, C.K.: *Kor. J. Pharmacogn.* **10**, 69 (1979).
8. Shin, K.H. and Woo, W.S.: *Korean Biochem. J.* **18**, 9 (1985).
9. Cook, L., Toner, J.J. and Fellows, E.J.: *Biochem. Pharmacol.* **111**, 131 (1954).
10. Kato, R., Chiera, E. and Vassanelli, P.:*Biochem Pharmacol.* **13**, 69 (1964).
11. Adamson, R.H. and Fouts, J.R.: *J. Pharm.Exp. Ther.* **127**, 87 (1959).
12. Teitel, S., O'Brien, J. and Brossi, A.: *J. Org. Chem.* **37**, 3368 (1972).