Isolation of Hepatic Drug Metabolism Inhibitors from the Seeds of *Myristica fragrans**

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Abstract □ The hexane extract from Nutmeg, the seed of *Myristica fragrans* significantly inhibited hepatic drug-metabolizing enzyme activity. Through systematic fractionation by SiO_2 column and vacuum liquid chromatography monitoring by bioassay, three components, myristicin, (I), licarin-B (II) and dehydrodiisoeugenol (III) were isolated as active principles. Compounds II and III, with a single treatment (200mg/kg, i.p.) showed not only a significant prolongation of hexobarbital-induced sleeping time but also a significant inhibition of aminopyrine N-demethylase and hexobarbital hydroxylase activities in mice. Compounds I and II provoked a sleep episode at a subhypnotic dose of HB, suggesting that they possess CNS-depressant properties.

Keywords □ nutmeg, *Myristica fragrans,* Myristicaceae, myristicin, licarin-B, dehydrodiisoeugenol, drug-metabolizing enzyme inhibitors.

During the course of our screening search for herbal medicines with hepatic drug-metabolizing enzyme (DME) modifying activities, we examined the effect of the methanol extracts of 150 medicinal plants on hexobarbital (HB)-induced sleeping time and strychnine mortality in mice. As the result, at least 30% of the herbal medicines tested were found to cause significant alterations in hepatic DME system.[2-4] Among them, nutmeg, the seed of *Myristica fragrans* being used not only for an aromatic stomachics in traditional Chinese medicine but for flavor, spice and condiment was revealed to cause marked alterations in HB-induced narcosis suggesting the presence of hepatic DME modifiers.[3,4] This paper deals with the isolation and characterization of the active principles and their effects on drug metabolism *in vivo* and *in vitro*.

EXPERIMENTAL

Plant materials

The seeds of *M. fragrans* were purchased from a local market and botanically identified. A voucher specimen is deposited at this Institute.

Instruments

The melting points were taken on a Mitamura Riken (No. 4204) melting point apparatus and uncorrected. ^{1}H-NMR and ^{13}-C-NMR spectra were taken with a Varian FT-80A NMR spectrometer. EI-MS spectra was determined on a Hewlett Packard (5985B) GC/MS spectrometer. Thin layer chromatography was performed using silica gel G (E. Merck 7729), column chromatography using 70-230 mesh silica gel 60 (E. Merck 7734) and vacuum liquid chromatography[5] using silica gel G (E. Merck 7731 for TLC).

Extraction and fractionation

The dried seeds were powdered and extracted 5 times with 95% methanol at room temperature for 4-5 days. The methanol extract was concentrated under reduced pressure and fractionated into hexane, neutral, phenolic, acidic and basic fractions as illustrated in Scheme 1.

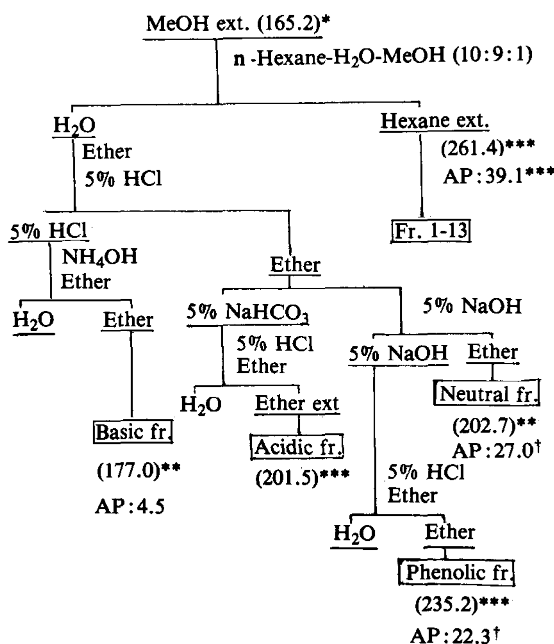
Isolation of compounds

Column chromatography of the hexane fraction over silica gel (hexane-benzene, gradient elution) gave 13 subfractions. Preparative VLC of subfractions 1 and 2 with hexane gave compound I as colorless oil (350 mg). MS m/z (%): 192(M^+, 100), 177($M-CH_3$, 5), 165($M-CH=CH_2$. 30). IR ν^{nujol}_{max} cm^{-1}: 1630, 1500, 1426 (aromatic-ring), 1445, 1345, 1305, 1270, 1230, 1031, 920(aromatic ether).

*Part 12 in the series "Studies on crude drugs acting on drug-metabolizing enzymes." For part 11 see ref 1.

^{1}H-NMR ($CDCl_3$) δ : 3.30(2H, br. d. $J=6.1$Hz, H-α), 3.85(3H, s, OCH_3), 4.90-5.10(2H, m, H-γ), 5.6-6.1 (1H, m, H-β), 5.87(2H, s, O-CH_2-O), 6.28 (2H, br. s. aromatic H). Preparatice VLC of subfractions 3-5(elution with hexane-ether = 10:1) gave compound II, which was recrystallized from hexane-ether (1:1) to afford colorless plates (234 mg). mp. 88-92°. MS m/z (%). 324(M^+, 83.9), 309(M-CH_3, 7.7), 202(M-$C_7H_6O_2$, 14.9), 189(M-$C_8H_7O_2$, 28.8), 135($C_8H_7O_2$, 100). UV(MeOH) λ_{max} nm(log ε): 220(4.34), 274(4.07). IR ν_{max}^{KBr} cm^{-1}: 1610, 1505, 1490, 1445(aromatic ring), 1395, 1250, 1030, 930(aromatic ether). ^{1}H-NMR (in $CDCl_3$) δ : 1.37(3H, d, J=6.8Hz, 3-CH_3), 1.86 (3H, d, J=5.4Hz, H-γ), 3.41(1H, dq, J=6.8 & 8.9 Hz, H-3), 3.89(3H, s, OCH_3), 5.10 (1H, d, J=8.9 Hz, H-2), 5.94(2H, s, O-CH_2-O), 6.04(1H, dq, J= 15.9 & 5.4Hz, H-β), 6.38(1H, d, J=15.9, H-α), 6.74-6.92(5H, aromatic H). ^{13}C-NMR ($CDCl_3$) δ : 18.0(3-Me), 18.2(C-γ), 45.8(C-3), 56.1(OMe), 93.3 (C-2), 101.1(O-CH_2O), 106.8(C-2′), 108.1(C-5′), 109.9(C-6), 113.5(C-4), 120.1(C-6′), 123.4(C-β), 131.1(C-α), 132.3(C-5), 133.3(C-3a), 134.5(C-1′), 144.3(C-7), 146.2(C-7a), 147.6(C-4′), 147.9(C-3′).

Preparative VLC of subfraction 6 (elution with hexane-ether = 10:3) gave compound III, which was recrystallized from hexane-benzene (1:1) to yield colorless needles (250 mg). MP. 91-93°. MS m/z (%): 326(M^+, 100), 311(M-CH_3, 5.3), 202(M-$C_7H_8O_2$, 22), 189(M-$C_8H_9O_2$, 13.6), 137($C_8H_9O_2$, 56.1); UV (MeOH) λ_{max} nm(log ε): 220(4.58), 277 (4.27). IR ν_{max}^{KBr} cm^{-1}: 3440(OH), 1630, 1520, 1500, 1455 (aromatic ring), 1220, 1030(aromatic ether). ^{1}H-NMR ($CDCl_3$) δ : 1.38(2H, d, J=6.8Hz, 3-CH_3), 1.87(3H, d, J=5.3Hz, H-γ), 3.40(1H, m, H-3), 3.88(6H, s, $OCH_3\times2$), 5.10(1H, d, J=9.2 Hz, H-2), 6.18(1H, dq, J=16.0 & 5.3Hz, H-β), 6.40(1H, d, J=16.0Hz, H-α), 6.77-6.96(5H, aromatic H). ^{13}C-NMR ($CDCl_3$) δ : 17.7(3-Me), 18.3(C-γ), 45.7(C-3), 56.1(OMe), 93.7(C-2), 109.2 (C-2′), 109.9(C-6), 113.5(C-4), 114.3(C-5′), 119.9 (C-6′), 123.4(C-β), 131.1(C-α), 132.2(C-1′), 132.3(C-5), 133.4(C-3a), 144.2(C-7), 145.9(C-4′), 146.6(C-3′), 146.8(C-7a).



Scheme I. Fractionation of MeOH extract of nutmeg monitoring by bioassays.

Figures in parentheses: sleeping time prolongation % of control. Control sleeping time: 27 min at hexobarbital-Na 50 mg/kg, i.p. AP = aminopyrine N-demethylase inhibition %, †$p < 0.1$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Sample dose: 500 mg/kg ip: for sleeping time: 200 mg/kg ip. for AP.

Biological test

Measurement of HB-induced sleeping time and enzyme assays were carried out as previously described.[13,14]

RESULTS AND DISCUSSION

A single i.p. administration of the methanol extract of the seeds of *M. fragrans* to mice showed a significant prolonging effect on HB-induced sleep-

Table I. Effects of nutmeg constituents on HB-induced hypnosis and AP N-demethylase activities *in vivo*

Treatment	Dose (mg/kg, ip)	HB hypnosis (min)$^{a)}$	AP N-demethylase (μ moles/30 min/g prot)$^{b)}$
Control	0.5% CMC	23.8 ± 2.6	14.13 ± 2.67
Myristicin	300	71.4 ± 12.7** (300.0)	7.01 ± 0.87** (49.6)
Licarin-B	200	46.5 ± 1.7** (195.4)	9.72 ± 1.19** (68.8)
Dehydro-diisoeugenol	200	35.9 ± 5.1* (150.8)	8.57 ± 0.81** (60.7)

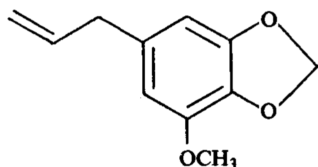
Mice were treated 30 min before injection of HB (50 mg/kg i.p.) and estimation of enzyme activity. Figures in parentheses indicate % of control. Significantly different from the control: *$p < 0.05$, **$p < 0.01$.

a) mean ± S.E. of 5 animals.

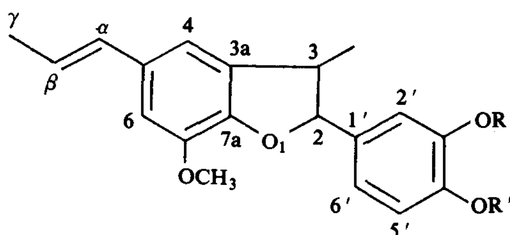
b) mean ± S.E. of 3 determinations.

ing time. In order to isolate the active principles, the methanol extract was fractionated, monitoring with prolongation of sleeping time as well as inhibition of aminopyrine (AP) N-demethylase activity *in vivo*.

As shown in Scheme 1, the most potent activities were observed in hexane fraction. A weaker but significant activity was also observed in both phenolic and neutral fractions. The hexane fraction was subjected to silica gel column chromatography. Preparative vacuum liquid chromatography (VLC)[5] of active subfractions gave compounds I, II and III, which were identified by their spectral data as myristicin, licarin B and dehydrodiisoeugenol, respectively, which were previously isolated from this plant[6-7] and *Licaria aritu*[8]. Table I shows the effect of a single treatment of the active principles isolated on HB-induced sleeping time and AP N-demethylase activity in mice.



Comp. I.



Comp. II. R + R' = CH_2

Comp. III. R = Me; R' = H

Table II. Effect of nutmeg constituents on subhypnotic dose of HB in mice

Treatments	Dose (mg/kg, i.p.)	Duration of sleep (Min ± S.E.)	
Control	0.5% CMC	0 ± 0	(0/5)
Myristicin	300	47.5 ± 2.5	(5/5)
Dehydro-diisoeugenol	200	10.0	(1/5)
Licarin-B	200	42.3 ± 16.8	(5/5)

Mice were injected with 40 mg/kg of hexobarbital-Na 30 min post sample treatment. Figures in parentheses represent No. of animals slept/No. of animals dosed.

Table III. Inhibitory potency of nutmeg constituents on HB hydroxylase and AP N-demethylase activity

Compound	IC_{50} value (M × 10^{-4})	
	HB hydroxylation	AP N-demethylation
Licarin B	7.24	1.99
Dehydrodiiso-eugenol	9.81	2.78

IC_{50} values were calculated from regression equation obtained by plots of relative velocity (velocity of the control / velocity in the presence of an inhibitor) versus concentration of the inhibitor.

It was shown that all three components caused a marked prolongation of HB-induced sleeping time as well as a significant inhibition of AP N-demethylase activity. Interestingly both compounds I and II provoked a distinct sleeping episode even at a subhypnotic dose of HB. At this dose control animals showed a severe ataxia but none of animals fell asleep (Table II). Compound III, however, exhibited very weak activity and only one animal slept among mice tested. The results strongly suggested that compounds I and II also have CNS depressant activity[9]. We previously reported that macelignan and piperine which have methylenedioxy moiety elicited a significant interaction with hepatic DME system.[10-11] Methylenedioxybenzenes such as piperonyl butoxide have been demonstrated as strong hepatic DME inhibitors[12]. It can, therefore, be well postulated that methylenedioxy moiety in compound, I and II may play important roles in the interaction with DME system. This tendency was also observed in the inhibitory potency of aminopyrine and HB metabolism *in vitro*. As shown in Table III, the inhibitory potency for AP N-demethylase and HB-hydroxylase of compound II was higher than that of compound III.

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