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Biotransformation of myrislignan by rat liver microsomes *in vitro*

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Abstract

Myrislignan (**1**), *erythro*-(1*R*,2*S*)-2-(4-allyl-2,6-dimethoxyphenoxyl)-1-(4-hydroxy-3-methoxyphenyl) propan-1-ol, is a major acyclic neolignan in seeds of *Myristica fragrans*. Studies have suggested that myrislignan may deter feeding activity, but little is known about its metabolism. We investigated the biotransformation of myrislignan by rat liver microsomes *in vitro*. Seven metabolites were produced by liver microsomes from rats pre-treated with sodium phenobarbital. These were identified, using spectroscopic methods, as myrislignanometins A–G (**2–8**), respectively.

Keywords: *Myristica fragrans*; Myristiceae; Biotransformation; Neolignan; Myrislignan; Erythro-(1*R*,2*S*)-2-(4-allyl-2,6-dimethoxyphenoxyl)-1-(4-hydroxyl-3-methoxyphenyl) propan-1-ol; Myrislignanometins A–G

1. Introduction

Myrislignan (**1**), *erythro*-(1*R*,2*S*)-2-(4-allyl-2,6-dimethoxyphenoxyl)-1-(4-hydroxy-3-methoxyphenyl) propan-1-ol, is a typical acyclic neolignan found in either seeds of nutmeg (Isogai et al., 1973) or the aril (mace) (Hattori et al., 1986) of *Myristica fragrans* Houtt. (Myristicaceae). Since it was discovered in 1973, it has attracted increasing interest because of its biological activities. Various studies have suggested that it may affect hepatic mixed function oxidase enzyme activity (Shin and Woo, 1990), deter feeding activity against silkworm larvae (Isogai et al., 1973), and have antifungal properties (Miyazawa et al., 1996). Two patents have been approved for its use as neoplasm and vascular smooth muscle contraction inhibitors (Matsumoto et al., 1991; Nakajima et al., 1999). Despite its varied biological activities, little is known about its metabolism. The biotransformation of myrislignan (**1**) in rats has been investigated and only one metabolite was found using both *in vitro* and *in vivo* approaches (Kasahara et al., 1995b). In addition, further studies suggested that some lignans and neolignans originating from plants can be chemically transformed by human intestinal bacteria and mammalian liver microsomes (Yang, 2006). Thus, the specific aim of study reported here was to examine the biotransformation of myrislignan (**1**) in liver microsomes from rats treated with the P450-inducing agent, phenobarbital. The metabolites were characterized by various methods including nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS).

2. Result and discussion

Myrislignan (**1**) biotransformation was investigated by its incubation of with liver microsomes from sodium phenobarbital pre-treated male Sprague–Dawley rats *in vitro*. The preliminary experiments clearly indicated that the concentration of **1** in the incubation system was remarkably decreased and it was transformed into several metabolites by the microsomes. Once the experimental conditions (reaction times and concentrations of **1**, microsomes and

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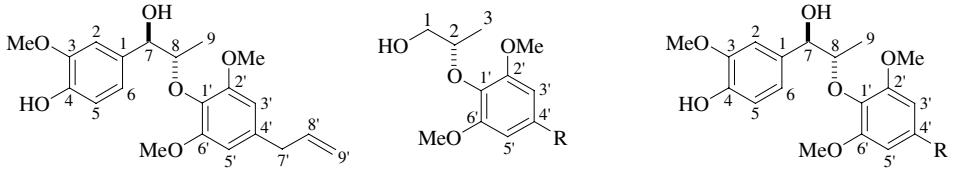
NADPH) were optimized (Yao and Wang, 1998), the EtOAc extract of the biotransformation products was subjected to silica gel column chromatography and semi-prep. reversed phase HPLC to give the seven metabolites **2**–**8** (named myrislignanometins A–G) and the primary compound **1**. The structures of the metabolites were determined by spectroscopic methods as shown in Fig. 1.

Myrislignanometin A (**2**) was isolated as an oily substance. Its high resolution EI mass spectrometry (HREIMS) displayed a $[M]^+$ ion peak at *m/z* 252.1363 (calcd. 252.1362), suggesting a molecular formula of $C_{14}H_{20}O_4$. The IR spectrum of **2** showed absorption bands for hydroxyl, olefinic, aromatic and methyl functions at 3471, 1638, 1608, 1590, 1502 and 1378 cm^{-1}, respectively. Comparison of the NMR spectroscopic data (Tables 1 and 2) of **2** with those of **1** indicated that the main differences were a lack of signals for the 4-hydroxy-3-methoxyphenyl group in **2** and a substitution of a hydroxymethinyl (–CHOH) group in **1** by a hydroxymethyl ($–CH_2OH$) group in **2**. These data indicated that **2** was a metabolite produced by oxidative cleavage of the 4-hydroxy-3-methoxyphenyl group from the structure of **1** and the transformation of the hydroxymethinyl group in **1** into the hydroxymethyl group in **2**. Its optical rotation value $[[\alpha]_D^{20} - 20.0$ ($CHCl_3$; *c* 1.5)] and comparison with that of (*R*)-2-[2,6-dimethoxy-4-((*E*)-prop-1-enyl)phenoxy] propanal $[[\alpha]_D^{25} + 37.9$ ($CHCl_3$; *c* 1.0)] and (*R*)-2-(4-allyl-2,6-dimethoxy-phenoxy) propanal $[[\alpha]_D^{25} + 30.6$ ($CHCl_3$; *c* 1.0)] (Curti et al., 2006), led us to conclude that the absolute configuration of the chiral carbon was in the *S*-form. Therefore, structure **2** was determined as (*S*)-(−)-2-(4-allyl-2,6-dimethoxyphenoxy)-1-propanol and named myrislignanometin A.

Myrislignanometin B (**3**) was isolated as an oily substance, with a molecular formula of $C_{14}H_{20}O_5$ determined by HREIMS. Its IR spectrum showed absorption bands for hydroxyl, olefinic, aromatic and methyl functions at 3366, 1638, 1604, 1502 and 1378 cm^{-1}, respectively. Comparison of ^1H and ^{13}C NMR spectroscopic data (Tables 1 and 2) of **3** with those of **2**, indicated that the characteristic signals of the allyl group were not found in **3**, but that the resonances of an additional 3-hydroxy-(*E*)-prop-1-enyl group were present instead. From its optical rotation ($[\alpha]_D^{20} - 30.3$) and comparison with that of (*R*)-2-[2,6-dimethoxy-4-((*E*)-prop-1-enyl)phenoxy] propanal, (*R*)-2-(4-allyl-2,6-dimethoxyphenoxy) propanal (Curti et al., 2006) and **2**, it was concluded that the absolute configuration of the chiral carbon was in the *S*-form. Thus, structure **3** was assigned as *S*-(−)-2-[4-(3-hydroxy-(*E*)-prop-1-enyl)-2,6-dimethoxyphenoxy]-1-propanol and named myrislignanometin B.

Myrislignanometin C (**4**) was obtained as an oily substance, with $[\alpha]_D^{20} - 38.8$ ($CHCl_3$; *c* 0.6). The molecular formula of **4** was deduced as $C_{14}H_{20}O_5$ from its high resolution ESI mass spectrometry (HRESIMS), which was in agreement with the EIMS and ^{13}C NMR spectroscopic data. The IR spectrum of **4** also showed absorption bands for hydroxyl, olefinic, aromatic and methyl functions at 3360, 1638, 1601, 1502 and 1380 cm^{-1}, respectively. Comparison of the ^1H and ^{13}C NMR spectroscopic data (Tables 1 and 2) of **4** with those of **3**,



1

2 R = allyl
3 R = 3-hydroxy-(*E*)-prop-1-enyl
4 R = 3-hydroxy-(*Z*)-prop-1-enyl
5 R = 1-hydroxyallyl
6 R = 3-hydroxy-(*E*)-prop-1-enyl
7 R = 3-hydroxy-(*Z*)-prop-1-enyl
8 R = 1-hydroxyallyl

Fig. 1. Structures of **1**–**8**.

Table 1
^1H NMR spectroscopic data for **2**–**5** (500 MHz, in CD_3OD)

H	2	3	4	5
1	3.55 *m*	3.57 *m*	3.56 *m*	3.56 *m*
2	4.11 *qd* (2.0, 6.0)	4.15 *qd* (1.5, 6.5)	4.17 *qd* (1.5, 6.5)	4.14 *qd* (1.5, 6.5)
3	1.25 *d* (6.0)	1.27 *d* (6.5)	1.27 *d* (6.5)	1.26 *d* (6.5)
3′	6.50 *s*	6.73 *s*	6.56 *s*	6.68 *s*
5′	6.50 *s*	6.73 *s*	6.56 *s*	6.68 *s*
7′	3.33 *d* (6.5)	6.54 *dt* (1.5, 16.0)	6.50 *dt* (1.5, 12.0)	5.07 *d* (6.0)
8′	5.96 *ddt* (6.5, 11.0, 17.0)	6.31 *dt* (6.0, 16.0)	5.80 *dt* (6.5, 12.0)	6.00 *ddd* (6.0, 11.0, 17.0)
9′	5.04 *dq* (1.5, 11.0) 5.08 *dq* (1.5, 17.0)	4.21 *dd* (1.5, 6.0)	4.35 *dd* (1.5, 6.5)	5.13 *dt* (1.5, 11.0) 5.30 *dt* (1.5, 17.0)
2′-OMe	3.81 *s*	3.84 *s*	3.83 *s*	3.83 *s*
6′-OMe	3.81 *s*	3.84 *s*	3.83 *s*	3.83 *s*

Table 2
^{13}C NMR spectroscopic data for **2**–**5** (125 MHz, in CD_3OD)

C	2	3	4	5
1	66.4	66.4	66.5	66.4
2	80.8	80.9	80.9	80.9
3	17.3	17.0	17.3	17.3
1′	135.2	134.6	134.1	136.1
2′	154.7	154.8	154.5	154.7
3′	106.8	104.7	107.4	104.7
4′	137.6	137.8	136.5	140.8
5′	106.8	104.7	107.4	104.7
6′	154.7	154.8	154.5	154.7
7′	41.4	131.5	132.2	76.1
8′	138.8	129.6	131.7	142.1
9′	116.1	63.6	59.8	114.9
3′-OMe	56.5	56.5	56.6	56.5
5′-OMe	56.5	56.5	56.6	56.5

showed that the characteristic resonances of the 3-hydroxy-(*E*)-prop-1-enyl group were not found in **4**, but that the resonances of an additional 3-hydroxy-(*Z*)-prop-1-enyl group were present instead. The absolute configuration of the chiral carbon of **4** was the same *S*-form as for **3**. On the basis of above analysis, structure **4** was identified as *S*-(−)-2-[4-(3-hydroxy-(*Z*)-prop-1-enyl)-2,6-dimethoxyphenoxy]-1-propanol and named myrislignanometin C.

Myrislignanometin D (**5**) was isolated as an oily substance. Its molecular formula $C_{14}H_{20}O_5$ was deduced from a ion peak $[M]^+$ at *m/z* 268 in EIMS and NMR spectroscopic data (Tables 1 and 2), which was consistent with a quasimolecular ion peak $[M+Na]^+$ at *m/z* 291.1204 (calcd. for $C_{14}H_{20}NaO_5$, 291.1203) in positive HRESIMS. The IR spectrum of **5** showed absorption bands for hydroxyl, olefinic, aromatic and methyl functions at 3417, 1638, 1593, 1502 and 1380 cm^{-1}, respectively. The ^1H and ^{13}C NMR spectroscopic data (Tables 1 and 2) of **5** showed a close similarity to those in **2** except for the side chain linked to the C-4′, which implied that **5** contained a different side chain at the C-4′ position relative to **2**. An 1-hydroxyallyl group linked to the C-4′ position was elucidated from analysis of the NMR spectra of **5** and comparison with the NMR spectra of an authentic sample 1′-hydroxymyristicin and **2**. In the NMR spectra, the signal at δ_H 3.33 (2H, *d*, $J = 6.5$ Hz, H-7′) and δ_C 41.4 (C-7′) in **2** showed a downfield shift to δ_H 5.07 (1H, *d*, $J = 6.0$ Hz) and δ_C 76.1 in **5**, which suggested that the C-7′ position of **5** was substituted by a hydroxyl group instead of a proton in **2**. Comparing the NMR spectroscopic data of the 1-hydroxyallyl group with those of (*R*)-1-(4′-methyoxy-phenyl) prop-2-en-1-ol (Evans and Leahy, 2003) and 1-(*S*)-(4-methylsulfonylphenyl)-2-propen -1-ol (George et al., 2006), the absolute configuration of C-7′ was determined to be in the *R*-form. Combined with the structures of 2, 3 and 4, the structure of **5** can be defined as (2*S*,7′*R*)-2-[4-(1-hydroxyallyl)-2,6-dimethoxyphenoxy]-1-propanol and named myrislignanometin D.

Myrislignanometin E (**6**) was isolated as an oily substance, with a molecular formula of $C_{21}H_{26}O_7$ determined by positive HRESIMS. The IR spectrum of **6** showed absorption bands at 3366, 1638, 1608, 1464, 1420 and 1381 cm^{-1} assignable to hydroxyl, olefinic, aromatic and methyl functions, respectively. The NMR spectroscopic data (Tables 3 and 4) of **6** showed close similarity with those of **1** except for the data relating to the side chain linked to the C-4′, which implied that **6** contained a different side chain at the C-4′ position compared with **1**. Comparison of the NMR spectra of **6** with those of **3** led us to conclude that a 3-hydroxy-(*E*)-prop-1-enyl group was linked to the C-4′ position. The small coupling constant value ($J_{7,8} = 4.0$ Hz) and ^{13}C NMR chemical shifts of C-7 (δ_C 83.7), C-8 (δ_C 75.5) and C-9 (δ_C 13.7) indicated that **6** belonged to an *erythro* series (Besombes et al., 2003; Hattori et al., 1988,1987). On the basis of the optical rotation $[\alpha]_D^{20} - 106.6$ ($CHCl_3$; *c* 0.25), the absolute configuration of **6** was determined as 7*R* and 8*S*, in accordance with that of **1** (Kasahara et al., 1995a). Consequently, the structure of **6** was concluded to be *erythro*-(1*R*,2*S*)-2-[4-(3-hydroxy-(*E*)-prop-1-enyl)-2,6-dimethoxyphenoxyl]-1-(4-hydroxy-3-methoxyphenyl)propan-1-ol and named myrislignanometin E.

Table 3
^1H NMR spectroscopic data for **1** and **6**–**8** (500 MHz, in CD_3OD)

H	1	6	7	8
2	6.93 *d* (1.5)	6.94 *d* (2.0)	6.94 *d* (2.0)	6.93 *d* (2.0)
5	6.72 *d* (8.0)	6.73 *d* (8.0)	6.72 *d* (8.0)	6.72 *d* (8.0)
6	6.68 *dd* (1.5, 8.0)	6.69 *dd* (2.0, 8.0)	6.70 *dd* (2.0, 8.0)	6.67 *dd* (2.0, 8.0)
7	4.74 *d* (3.0)	4.74 *d* (4.0)	4.75 *d* (4.0)	4.74 *d* (2.5)
8	4.26 *qd* (3.0, 6.5)	4.30 *qd* (4.0, 6.5)	4.33 *qd* (4.0, 6.5)	4.29 *qd* (2.5, 6.5)
9	1.08 *d* (6.5)	1.10 *d* (6.5)	1.11 *d* (6.5)	1.09 *d* (6.5)
3′	6.51 *s*	6.75 *s*	6.58 *s*	6.71 *s*
5′	6.51 *s*	6.75 *s*	6.58 *s*	6.71 *s*
7′	3.33 *d* (7.0)	6.54 *dt* (1.5, 16.0)	6.51 *dt* (1.5, 12.0)	5.08 *d* (6.5)
8′	5.96 *ddt* (7.0, 10.0, 17.0)	6.32 *dt* (5.5, 16.0)	5.81 *dt* (6.5, 12.0)	6.01 *ddd* (6.5, 10.5, 17.0)
9′	5.05 *dq* (1.5, 10.0) 5.10 *dq* (1.5, 17.0)	4.22 *dd* (1.5, 5.5)	4.36 *dd* (1.5, 6.5)	5.14 *dt* (1.5, 10.5) 5.30 *dt* (1.5, 17.0)
3-OMe	3.79 *s*	3.82 *s*	3.82 *s*	3.82 *s*
2′-OMe	3.81*s*	3.85 *s*	3.84 *s*	3.84 *s*
6′-OMe	3.81*s*	3.85 *s*	3.84 *s*	3.84 *s*

Table 4
^{13}C NMR spectroscopic data for **1** and **6**–**8** (125 MHz, in CD_3OD)

C	1	6	7	8
1	133.5	133.5	133.5	133.5
2	111.0	111.1	111.3	111.1
3	148.7	148.7	148.7	148.8
4	146.7	146.6	146.8	146.7
5	115.8	115.7	115.7	115.7
6	120.1	120.9	120.2	120.1
7	83.7	83.7	83.8	83.8
8	75.5	75.5	75.6	75.5
9	13.8	13.7	13.7	13.8
1′	134.8	134.7	134.1	135.6
2′	154.7	154.8	154.9	154.7
3′	106.8	104.8	107.3	104.7
4′	137.8	137.9	136.4	140.8
5′	106.8	104.8	107.3	104.7
6′	154.7	154.8	154.9	154.7
7′	41.4	132.9	132.2	76.1
8′	138.8	129.6	131.7	142.0
9′	116.1	63.1	58.3	114.9
3-OMe	56.3	56.6	56.3	56.3
3′-OMe	56.6	56.6	56.6	56.6
5′-OMe	56.6	56.6	56.6	56.6

Myrislignanometin F (**7**) was isolated as an oily substance, with a molecular formula of $C_{21}H_{26}O_7$ determined by positive HRESIMS. The NMR spectroscopic data (Tables 3 and 4) of **7** showed close similarity with those of **6** except for those pertaining to the side-chain linked to C-4′, which implied that **7** contained a different side chain at the C-4′ position when compared to **6**. Comparison of the NMR spectra of **7** with those of **4** led us to conclude that a 3-hydroxy-(*Z*)-prop-1-enyl group was linked to the C-4′ position. Comparing the optical rotation, $J_{7,8}$ value and ^{13}C NMR chemical shifts of C-7, C-8 and C-9 with those of **6**, suggested that **7** had the absolute configuration of 7*R* and 8*S*, and belonged to an *erythro* series. On the basis of the above evidence, the structure of 7 was determined to be *erythro*-(1*R*,2*S*)-2-[4-(3-hydroxy-(*Z*)-prop-1-enyl)-2,6-dime-thoxyphenoxyl]-1-(4-hydroxy-3-methoxyphenyl) propan-1-ol and named myrislignanometin F.

Myrislignanometin G (**8**) was isolated as an oily substance. Its molecular formula $C_{21}H_{26}O_7$ was established by HRESIMS. The NMR data (Tables 3 and 4) of **8** showed close similarity with those of **1**, **6** and **7**, and only differences between them were signals due to a 1-hydroxyallyl group [^1H NMR: δ_H 5.08 (1H, *d*, $J = 6.5$ Hz), 5.14 (1H, *dt*, $J = 1.5$, 10.5 Hz), 5.30 (1H, *dt*, $J = 1.5$, 17.0 Hz), 6.01 (1H, *ddd*, $J = 6.5$, 10.5, 17.0 Hz); ^{13}C NMR: δ_C 76.1, 114.9, 142.0] which in the same way as for **5** was linked at the C-4′. The comparisons of the $J_{7,8}$ value and ^{13}C NMR chemical shifts of C-7, C-8 and C-9 with those of 6 and 8 led us conclude the 1,2-propanediol moiety of **8** to be an *erythro* series. According to the NMR data of the 1-hydroxyallyl group, the absolute configuration of C-7′ was determined to be in the *R*-form, which accorded with **5**. Combined with the structures of **6** and **7**, the structure of **8** was identified as *erythro*-(1*R*,2*S*,7′*R*)-2-[4-(1-hydroxyallyl)-2,6-dimethoxyphenoxyl]-1-(4-hydroxyl-3-methoxyphenyl) propan-1-ol and named myrislignanometin G.

In the above mentioned metabolites, myrislignanometins A–D (**2**–**5**) were fission products resulting from the cleavage of an aryl group and myrislignanometins E–G (**6**–**8**) were hydroxylation products from the primary compound. It is worth pointing out that myrislignanometins A–D (**2**–**5**) were derived from myrislignan (**1**) by fission of the carbon–carbon bond between C-1 and C-7. This type of fission has not previously been reported in the drugs metabolism (Yang et al., 2003b). Generally, this type of acyclic neolignans is transformed to other products via enzymic and non-enzymic oxidations by fission of the carbon–carbon bond between either C-7 and C-8 or the carbon-oxygen bond between C-8 and C-1′ (Crestini et al., 2004; Bohlin et al., 2005). We consider that it may first form a tricyclic ring oxide, followed by an intermediate with amphoteric ions, and that finally the intermediate rearranges to make the fission products (Zhong, 1996).

The myrislignanometins pairs, C/D and E/F were two pairs of isomers, and took *erythro* or *threo* forms at the 1,2-propanediol moiety. Therefore, it is important to determine the absolute configuration of the metabolites. In the present paper, we determined their absolute configuration using the coupling constant ($J_{H\text{-}7,H\text{-}8}$) value and their optical rotation. It is well known that it is difficult to separate chiral compounds with conventional HPLC stationary phases. Fortunately, all the isomers were successfully separated using a universal C_{18} column with a MeCN–H_2O mobile phase in this study.

3. Concluding remarks

It has been reported (Chan and Caldwell, 1992) that phenylpropenoids with allyl groups, and not propenyl groups, are hydroxylated at the 1′-carbon (the C-7′ position in this paper) by drug metabolism enzymes in hepatic microsomes and that the 1′-hydroxylated metabolite cause genotoxicity by forming adducts with DNA, such as methyleugenol (Gardner et al., 1996), safrole (De Vincenzi et al., 2000), estragole (Boberg et al., 1983), elemicin, α-asarone and β-asarone (Hasheminejad and Caldwell, 1994) in the liver. In this paper, two 1′-hydroxylated metabolites (**5** and **8**) were obtained from myrislignan (**1**), and these may have a similar genotoxicity. Nutmeg has been shown to have toxicological side-effects in clinical applications (Forrester, 2005; Green, 1959; Payne, 1963). It is generally thought this toxicity is similar to that caused by myristicin (Beyer et al., 2006; Hallstrom and Thuvander, 1997; Stein et al., 2001), elemicin and safrole (Beyer et al., 2006). There are also various other neolignans with an allyl group in nutmeg (Wang et al., 2004; Yang et al., 2003a) and mace (Hattori et al., 1986,1987; Hada et al., 1988; Isogai et al., 1973). The results of the present paper suggest that toxicity is not solely caused by myristicin, elemicin and safrole, but

is also relative to the presence of an allyl group in some of the neolignans.

4. Experimental

4.1. General experimental procedures

Optical rotations were measured on a Perkin–Elmer 243B polarimeter with $CHCl_3$ as the solvent whereas UV spectra were obtained on a Varian Cary-300 ultraviolet–visible photometer in MeOH solution. IR spectra were taken on a Thermo Nicolet Nexus 470 FT-IR spectrometer. Mass spectra were recorded on a Finnigan TRACE 2000 GC–MS spectrometer (for EIMS) and a Bruker DALTONICS APEX IV Fourier transform ICR high-resolution mass spectrometer (for HRESIMS and HR-EIMS). NMR spectra were recorded on a Varian INOVA-500 (500 MHz for ^1H NMR and 125 MHz for ^{13}C NMR) using CD_3OD as solvents, with TMS as int. standard. Semi-prep. HPLC was performed on a P680 chromatograph (Dionex Co., CA) equipped with an UVD170U detector using a Phenomenex PRODIGY ODS column (250 × 21.2 mm, 10 μm) at a flow rate 7.0 mL/min. Open cc was carried out using silica gel (200–300 mesh, Tsingdao Marine Chemical Co., Tsingdao, PR China) as stationary phase. TLC was conducted on silica gel GF_{254} plates (Merck).

Myrislignan (**1**) was isolated from a methanol extract of the seed of *M. fragrans* as described in a previous paper (Yang et al., 2003a). β-Nicotinamide adenine dinucleotide phosphate (NADP), reduced nicotinamide adenine dinucleotide (NADH), glucose-6-phosphate and glucose-6-phosphate dehydrogenase was purchased from Sigma (St. Louis, Mo, USA). Sodium phenobarbital (PB) was purchased from Peking University Third Hospital. All organic solvents and other chemicals were of the highest purity available.

4.2. Preparation of rat liver microsomes

Twenty male Sprague–Dawley rats (180–200 g body weight) obtained from the Department of Laboratory Animal Science, Peking University Health Science Center (Peking University, China) were group-housed and maintained under conventional conditions at 22 ± 1 °C in an alternating 12 h light/dark cycle, fed a standard laboratory chow and were allowed water *ad libitum*. To induce cytochrome P450 enzymes, rats were given PB in physiological saline intraperitoneally as a single dose of 80 mg/kg body weight and sacrificed on day 3 after treatment. Their livers were quickly removed and then washed with ice-cold 10 mM potassium phosphate buffer (pH 7.4), and kept ice-cold. The livers were sliced and thoroughly homogenized with four-volumes of potassium phosphate buffer (pH 7.4) using an F6/10 superfine homogenizer (Fluko equipment, Shanghai, China), and centrifuged at 12,500*g* for 15 min at 4 °C using a GL-20B centrifuge (Anke equipment, Shanghai, China) to produce a supernatant. The supernatant was transferred to ultracentrifuge tubes and then centrifuged at 105,000*g* for 65 min at 4 °C using an LB-80M ultracentrifuge (Beckman instrument, CA, USA). The microsomal pellets were resuspended in 50 mM potassium phosphate buffer (pH 7.4). The amount of microsomal protein was determined following the method of Lowry-Folin (Lowry et al., 1951). Cytochrome P450 concentrations were determined as described by (Omura and Sato, 1964). Hepatic microsomes were found to contain 1.65 nmol cytochrome P450 per mg protein.

4.3. Myrislignan biotransformation by rat liver microsomes

A typical 500 mL biotransformation incubation mixture consisted of 2.5 g protein (rat liver microsomes), 100 mM potassium phosphate buffer (pH 7.4) and 100 mg of myrislignan (**1**) as substrate. After preincubation for 5 min, the reaction was initiated by adding a NADPH generating system (1.0 mM NADP, 0.5 mM NADH, 10 mM glucose-6-phosphate, 1.0 IU/mL glucose-6-phosphate dehydrogenase and 4.0 mM $MgCl_2$). Incubations were carried out at 37 °C for 2 h with continuous shaking in a Dubnoff incubator. Reactions were terminated by adding ice-cold EtOAc (500 mL), followed by centrifugation at 4 °C. The supernatants were evaporated to dryness under N_2 gas to give residues.

4.4. Extraction and isolation of biotransformation products

The residues of biotransformation products (630 mg) were redissolved in redistilled H_2O (500 mL) and extracted with EtOAc (3 × 500 mL). The combined organic extracts were evaporated under reduced pressure at 37 °C to afford a residue (450 mg), with the latter applied to a silica gel column (Ø 2 × 30 cm, silica gel, 200–300 mesh, 20 g) eluted with a gradient of *N*-hexane-EtOAc → MeOH. The eluant was collected in 10 mL portions to give six fractions: fraction 1 (182.1 mg; *N*-hexane/EtOAc = 10:1, *v*/*v*, 100 mL), 2 (30.2 mg; *N*-hexane/EtOAc = 85:15, *v*/*v*, 150 mL), 3 (51.4 mg; *N*-hexane/EtOAc = 70:30, *v*/*v*, 200 mL), 4 (15.1 mg; *N*-hexane/EtOAc = 50:50, *v*/*v*, 200 mL)], 5 (15.4 mg; EtOAc, 100 mL) and 6 (124.8 mg; MeOH, 100 mL).

Fractions 2 and 3 were purified by HPLC with MeOH–H_2O (3:1) as mobile phase, to afford myrislignan (**1**, 50 mg) and myrislignanometin A (**2**, 3.0 mg), respectively. Fractions 4 and 5 were purified by HPLC with MeOH–H_2O (1:1) as mobile phase, to afford myrislignanometin B (**3**, 2.2 mg), and sub-fractions 5–1 (5.0 mg) and 5–2 (6.5 mg), respectively. Sub-fraction 5–1 were purified by HPLC with MeCN–H_2O (4:6) as mobile phase to give myrislignanometins C (**4**, 1.2 mg) and D (**5**, 1.2 mg). Sub-fractions 5–2 were purified using the same conditions as for sub-fraction 5–1 to afford myrislignanometins E (**6**, 0.5 mg), F (**7**, 1.0 mg) and G (**8**, 1.5 mg).

*4.4.1. Myrislignan (erythro-(1R,2S)-2-(4-allyl-2,6-dimethoxyphenoxyl)-1-(4-hydroxy-3-methoxyphenyl) propan-1-ol, **1**)*

Colorless prisms (hexane), m.p. 96–97 °C. The NMR, IR and EIMS data were similar to those reported in a previous paper (Kasahara et al., 1995b); the ^{1}H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD) data are given in Tables 3 and 4.

*4.4.2. Myrislignanometin A (S-(−)-2-(4-allyl-2,6-dimethoxyphenoxy)-1-propanol, **2**)*

Oily substance; $[\alpha]_D^{20}$ − 20.0 ($CHCl_3$; *c* 1.5); UV γ_{max}^{MeOH} nm (log ε): 269 (3.14); IR ν_{max}^{KBr} cm^{-1}: 3471, 2923, 2853, 1638, 1608, 1590, 1502, 1460, 1422, 1378, 1331, 1238, 1126, 1056, 992, 918, 864, 824, 789, 704, 605. For ^{1}H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD spectra), see Tables 1 and 2; EIMS *m/z* (rel. int.): 252 $[M]^+$ (65), 221 (11), 194 (100), 179 (42), 163 (35), 151 (25), 131 (40), 91 (40), 77 (26); HREIMS *m/z*: 252.1363 $[M]^+$ (calcd. for $C_{14}H_{20}O_4$, 252.1362).

*4.4.3. Myrislignanometin B (S-(−)-2-[4-(3-hydroxy-(E)-prop-1-enyl)-2,6-dimethoxyphen-oxy]-1-propanol, **3**)*

Oily substance; $[\alpha]_D^{20}$ − 30.3 ($CHCl_3$; *c* 0.55); UV γ_{max}^{MeOH} nm (log ε): 268 (3.16); IR ν_{max}^{KBr} cm^{-1}: 3366, 2924, 2854, 1638, 1604, 1502, 1461, 1378, 1240, 1124, 1049, 925, 863, 776, 722, 620. For ^{1}H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD spectra), see Tables 1 and 2; EIMS *m/z* (rel. int.): 268 $[M]^+$ (10), 237 (3), 210 (27), 194 (6), 179 (10), 167 (55), 155 (22), 137 (17), 123 (20), 111 (25), 83 (37), 69 (100), 55 (66); HREIMS *m/z*: 268.1315 $[M]^+$ (calcd. for $C_{14}H_{20}O_5$, 268.1311).

*4.4.4. Myrislignanometin C (S-(−)-2-[4-(3-hydroxy-(Z)-prop-1-enyl)-2,6-dimethoxyphen- oxy]-1 -propanol, **4**)*

Oily substance; $[\alpha]_D^{20}$ − 38.8 ($CHCl_3$; *c* 0.6); UV γ_{max}^{MeOH} nm (log ε): 260 (3.25); IR ν_{max}^{KBr} cm^{-1}: 3360, 2924, 2854, 1638, 1601, 1502, 1462, 1409, 1380, 1231, 1125, 1044, 924, 863, 775, 723, 611. For ^{1}H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD spectra), see Tables 1 and 2; EIMS *m/z* (rel. int.): 268 $[M]^+$ (42), 237 (8), 210 (100), 194 (6), 179 (45), 167 (29), 155 (91), 137 (23), 123 (61), 111 (48), 83 (51), 69 (77), 55 (91); positive HRESIMS *m/z*: 291.1205 $[M+Na]^+$ (calcd. for $C_{14}H_{20}NaO_5$, 291.1203).

*4.4.5. Myrislignanometin D ((2S,7′R)-2-[4-(1-hydroxyallyl)-2,6-dimethoxyphenoxy]-1- propanol, **5**)*

Oily substance; $[\alpha]_D^{20}$ 0 ($CHCl_3$; *c* 1.1); UV γ_{max}^{MeOH} nm (log ε): 270 (3.17); IR ν_{max}^{KBr} cm^{-1}: 3417, 2925, 2854, 1638, 1593, 1502, 1461, 1420, 1380, 1326, 1229, 1123, 1059, 989, 923, 835, 754, 723, 622. For ^{1}H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD spectra), see Tables 1 and 2; EIMS *m/z* (rel. int.): 268 $[M]^+$ (26), 237 (8), 210 (95), 194 (10), 179 (38), 167 (25), 155 (94), 137 (16), 123 (50), 111 (12), 95 (27), 69 (18), 55 (100); positive HRESIMS *m/z*: 291.1204 $[M+Na]^+$ (calcd. for $C_{14}H_{20}NaO_5$, 291.1203).

*4.4.6. Myrislignanometin E (erythro-(1R,2S)-2-[4-(3-hydroxy-(E)-prop-1-enyl)-2,6-di-methoxyphenoxyl]-1-(4-hydroxyl-3-methoxyphenyl) propan-1-ol, **6**)*

Oily substance; $[\alpha]_D^{20}$ − 106.6 ($CHCl_3$; *c* 0.25); UV γ_{max}^{MeOH} nm (log ε): 271 (3.62); IR ν_{max}^{KBr} cm^{-1}: 3366, 2924, 2853, 1638, 1608, 1464, 1420, 1381, 1226, 1124, 1042, 929, 862, 829, 778, 620. For ^{1}H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD spectra), see Tables 3 and 4; EIMS *m/z* (rel. int.): 372 $[M-H_2O]^+$ (1), 237 (5), 210 (16), 194 (5), 182 (10), 149 (16), 137 (16), 111 (20), 83 (44), 69 (57), 55 (100); positive HRESIMS *m/z*: 413.1575 $[M+Na]^+$ (calcd. for $C_{21}H_{26}NaO_7$, 413.1571).

*4.4.7. Myrislignanometin F (erythro-(1R,2S)-2-[4-(3-hydroxy-(Z)-prop-1-enyl)-2,6-di-methoxyphenoxyl]-1-(4-hydroxyl-3-methoxyphenyl) propan-1-ol, **7**)*

Oily substance; $[\alpha]_D^{20}$ − 100.0 ($CHCl_3$; *c* 0.50); UV γ_{max}^{MeOH} nm (log ε): 260 (3.68); IR ν_{max}^{KBr} cm^{-1}: 3370, 2925, 2854, 1595, 1501, 1462, 1423, 1379, 1236, 1124, 1042, 928, 861, 828, 774, 619. For ^{1}H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD spectra), see Tables 3 and 4; EIMS *m/z* (rel. int.): 372 $[M-H_2O]^+$ (1), 237 (7), 210 (57), 194 (11), 182 (30), 149 (32), 137 (23), 123 (22), 111 (26), 83 (42), 69 (55), 55 (100); positive HRESIMS *m/z*: 413.1573 $[M+Na]^+$(calcd. for $C_{21}H_{26}NaO_7$, 413.1571).

*4.4.8. Myrislignanometin G (erythro-(1R,2S,7′R)-2-[4-(1-hydroxyallyl)-2,6-dimethoxy-phenoxyl]-1-(4-hydroxyl-3-methoxyphenyl) propan-1-ol, **8**)*

Oily substance; $[\alpha]_D^{20}$ ($CHCl_3$; *c* 0.75); UV γ_{max}^{MeOH} nm (log ε): 278 (3.61); IR ν_{max}^{KBr} cm^{-1}: 3401, 2926, 2853, 1594, 1502, 1462, 1422, 1382, 1229, 1123, 1037, 992, 922, 831, 751, 618. For ^{1}H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD spectra), see Tables 3 and 4; EIMS *m/z* (rel. int.): 372 $[M-H_2O]^+$ (5), 237 (12), 210 (100), 194 (13), 180 (23), 155 (41), 149 (30), 137 (20), 123 (26), 111 (21), 95 (27), 69 (41), 55 (92); HRESIMS *m/z*: 413.1577 $[M+Na]^+$ (calcd. for $C_{21}H_{26}NaO_7$, 413.1571).

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