# Ovafolinins A—E, Five New Lignans from *Lyonia ovalifolia*

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**Five new lignans, ovafolinins A—E (1—5), were isolated from the wood of *Lyonia ovalifolia* (Ericaceae). The structures of 1—5 were elucidated based on 2D NMR spectroscopy, X-ray crystallography, and other chemical methods.**

**Key words** *Lyonia ovalifolia*; Ericaceae; lignan; benzoxepin structure

Lignans are a widely distributed class of dimeric phenylpropanoids, and are one of the major classes of phytoestrogens[1] that are known to alleviate menopausal symptoms and lower the risk of cardiovascular disease.[2] Furthermore, they also possess anticancer[3] and antiviral properties,[4] inhibit certain enzymes[5] and exhibit antioxidant activity.[6] In the present study, we targeted lignans from *Lyonia ovalifolia* var. elliptica, a deciduous tree distributed in Taiwan, China and Japan. Yasue *et al.* identified lyoniols-A, -B, and -C to be toxic components isolated from the leaves,[7] and lyoniside, which is a major component and lyoniresinol in the bark of this plant.[8,9] In addition, Sakakibara *et al.* identified triterpene glycosides in this plant.[10,11] Herein, we report on five novel lignans, ovafolinins A (**1**)—E (**5**), isolated from the wood of *L. ovalifolia*. Furthermore, we have identified ovafolinins A (**1**)—C (**3**) to bear a unique benzoxepin structure.

## Results and Discussion

The filtrate of the aqueous EtOH extract of *L. ovalifolia* afforded new five lignans named ovafolinin A (**1**), B (**2**), C (**3**), D (**4**), and E (**5**) along with lyoniside (**6**) and lyoniresinol (**7**) as known compounds. The ovafolinins were isolated by successive column chromatography on highly porous synthetic resin (Diaion HP-20), subsequent silica gel chromatography, and finally preparative reversed-phase HPLC.

Ovafolinin A (**1**) was obtained as a colorless prisms. The molecular formula was determined to be $C_{22}H_{24}O_8$ by HR-electrospray ionization (ESI)-MS, which showed a $[M+H]^+$ peak at *m/z* 417.1523 (Calcd for $C_{22}H_{25}O_8$, 417.1549). The IR spectrum showed absorptions for hydroxyl groups (3392 $cm^{-1}$) and aromatic rings (1615 $cm^{-1}$). The UV absorption maxima occurred at 243 and 283 nm, implying the presence of conjugated double bond systems in the molecule. The $^1$H-NMR spectrum showed signals for four methoxyl groups ($\delta$ 3.23, 3.77, 3.86, 4.06) and two aromatic protons ($\delta$ 6.26, 6.52) (Table 1). The $^{13}$C-NMR spectrum showed signals for four methoxyl carbons ($\delta$ 55.8, 56.0, 59.1, 60.6), two methylene carbons ($\delta$ 69.3, 72.5), six methine ($\delta$ 37.4, 39.7, 43.0, 79.0, 100.7, 104.8), and ten quaternary carbons ($\delta$ 122.9, 124.5, 128.7, 135.0, 138.6, 143.9, 144.9, 145.7, 146.1, 152.0) (Table 2). The $^1$H–$^1$H correlation spectroscopy (COSY), heteronuclear multiple quantum correlation (HMQC), and heteronuclear multiple bond correlation (HMBC) spectra suggested **1** was an aryltetralin-type lignan. The $^1$H–$^1$H correlations were observed between H-7 and H-8, H-8 and H-9, H-8 and H-8′, H-7′ and H-8′, and H-8′ and H-9′. The strong HMBC correlations of H-1 with C-2, C-3, C-5, C-6 and C-7 and of H-7′ with C-4 suggests that **1** contains the A ring of an aryltetralin-type lignan. The existence of a B ring was confirmed by the HMBC correlations of H-7 with C-1, C-5, C-6, C-8, C-9 and C-9′ and of H-7′ with C-1′, C-8′, and C-9′. The C ring was suggested by the HMBC correlations of H-3′ with C-1′, C-2′, and C-5′ (Fig. 2). One of methoxyl groups is linked to C-4′ based on the HMBC correlations of the methoxyl protons ($\delta$ 3.23) with C-4′ ($\delta$ 144.9). Another methoxyl group is connected to C-2 based on the HMBC correlation of H-1 and the methoxyl protons ($\delta$ 3.86) with C-2 ($\delta$ 146.1). The HMBC correlations of the third methoxyl protons ($\delta$ 3.77) indicated connection to C-6′ ($\delta$ 145.7). The position of the fourth methoxyl group is suggested to be at C-4 by HMBC correlations of methoxyl protons ($\delta$ 4.06) and H-7′ with C-4 ($\delta$ 143.9). These methoxyl group assignments were confirmed by NOE correlations of H-1 with the methyl protons of $OCH_3$ at the positions 2, 4′, and 4, respectively. HMBC correlations of H-9 with C-2′ and of H-9′ with C-7, and the $^{13}$C-NMR chemical shifts indicate the existence of a methyleneoxy bridge between C-7 and C-9′ and an ether bonding between C-2′ and C-9. Based on the weak NOE correlations detected between H-1 and H-7, H-7

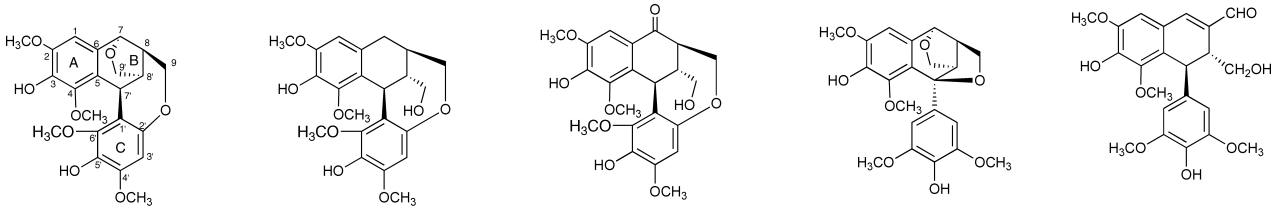


Fig. 1. Structures of Ovafolinins A (**1**)—E (**5**)

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Table 1. $^1$H-NMR (500 MHz) Spectral Data for **1**—**5** at 300 K[a]

| | **1**[b] | **2**[b] | **3**[c] | **4**[b] | **5**[c] |
|---|---|---|---|---|---|
| 1 | 6.52 (1H, s) | 6.38 (1H, s) | 7.34 (1H, s) | 6.63 (1H, s) | 6.99 (1H, s) |
| 7 | 4.75 (1H, br d, 4.4) | 2.86 (1H, br d, 17.4) | 4.82 (1H, d, 5.4) | 7.51 (1H, s) | |
| | | 3.03 (1H, dd, 7.1, 17.4) | | | |
| 8 | 2.32 (1H, *) | 2.24 (1H, *) | 2.76 (1H, *) | 3.10 (1H, *) | |
| 9 | 3.96 (1H, dd, 2.8, 13.3) | 3.82 (1H, *) | 3.90 (1H, *) | 3.48 (1H, br d, 9.3) | 9.49 (1H, s) |
| | 4.51 (1H, *br d, 13.3) | 4.41 (1H, dd, 2.7, 12.1) | 4.54 (1H, dd, 3.3, 11.9) | 4.24 (1H, dd, 6.4, 9.3) | |
| 2′ | | | | 7.10 (1H, d, 1.4) | 6.31 (1H, s) |
| 3′ | 6.26 (1H, s) | 6.25 (1H, s) | 6.33 (1H, s) | | |
| 6′ | | | | 6.43 (1H, d, 1.4) | 6.31 (1H, s) |
| 7′ | 4.49 (1H, d, 2.3) | 4.59 (1H, br s) | 4.98 (1H, br s) | | 4.80 (1H, s) |
| 8′ | 2.60 (1H, *) | 2.22 (1H, *) | 2.37 (1H, *) | 2.70 (1H, br t, 6.2) | 3.21 (1H, dd, 4.5, 10.2) |
| 9′ | 3.73 (1H, br d, 8.5) | 3.62 (1H, dd, 7.1, 10.6) | 3.51 (2H, *) | 3.86 (1H, br d, 9.8) | 3.12 (1H, t, 10.2) |
| | 4.11 (1H, dd, 2.7, 8.5) | 3.72 (1H, *) | | 3.99 (1H, dd, 6.2, 9.8) | 3.48 (1H, dd, 4.5, 10.2) |
| OMe-2 | 3.86 (3H, s) | 3.81 (3H, s) | 3.81 (3H, s) | 3.93 (3H, s) | 3.72 (3H, s) |
| OMe-4 | 4.06 (3H, s) | 4.06 (3H, s) | 3.93 (3H, s) | 3.95 (3H, s) | 3.96 (3H, s) |
| OMe-3′ | | | | 3.33 (3H, s) | 3.33 (3H, s) |
| OMe-4′ | 3.23 (3H, s) | 3.42 (3H, s) | 3.47 (3H, s) | | |
| OMe-5′ | | | | 3.76 (3H, s) | 3.72 (3H, s) |
| OMe-6′ | 3.77 (3H, s) | 3.74 (3H, s) | 3.75 (3H, s) | | |

*a*) *J*-Values are given in Hz in parentheses. *b*) In $CDCl_3$. *c*) In $CD_3OD$. * Multiplicity was not determined due to overlapping and/or broadening of the signals.

Table 2. $^{13}$C-NMR (125 MHz) Spectral Data for **1**—**5** at 300 K

| | **1**[a] | **2**[a] | **3**[b] | **4**[a] | **5**[b] |
|---|---|---|---|---|---|
| 1 | 104.8 | 105.4 | 104.2 | 106.6 | 109.7 |
| 2 | 146.1 | 146.1 | 149.0 | 145.4 | 149.5 |
| 3 | 138.6 | 136.3 | 147.0 | 139.9 | 145.3 |
| 4 | 143.9 | 144.6 | 146.9 | 145.4 | 147.9 |
| 5 | 122.9 | 122.4 | 125.7 | 121.1 | 127.0 |
| 6 | 128.7 | 126.1 | 132.9 | 126.7 | 123.9 |
| 7 | 79.0 | 29.3 | 200.4 | 81.2 | 149.0 |
| 8 | 43.0 | 33.9 | 51.6 | 48.9 | 136.4 |
| 9 | 69.3 | 80.2 | 79.1 | 64.1 | 194.3 |
| 1′ | 124.5 | 123.8 | 124.2 | 134.3 | 136.3 |
| 2′ | 152.0 | 152.8 | 153.6 | 101.7 | 106.1 |
| 3′ | 100.7 | 145.3 | 146.9 | 146.7 | 149.2 |
| 4′ | 144.9 | 136.3 | 137.3 | 133.3 | 135.0 |
| 5′ | 135.0 | 145.4 | 148.5 | 146.5 | 149.2 |
| 6′ | 145.7 | 101.1 | 102.4 | 100.2 | 106.1 |
| 7′ | 37.4 | 29.9 | 31.5 | 86.0 | 38.5 |
| 8′ | 39.7 | 43.4 | 46.8 | 58.4 | 43.6 |
| 9′ | 72.5 | 64.8 | 65.0 | 64.0 | 62.7 |
| OMe-2 | 56.0 | 55.7 | 56.5 | 56.3 | 56.6 |
| OMe-4 | 60.6 | 61.5 | 61.7 | 61.0 | 56.8 |
| OMe-3′ | | | | 61.0 | 60.7 |
| OMe-4′ | 59.1 | 59.6 | 60.2 | | |
| OMe-5′ | | | | 56.3 | 56.6 |
| OMe-6′ | 55.8 | 55.9 | 56.5 | | |

*a*) In $CDCl_3$. *b*) In $CD_3OD$.

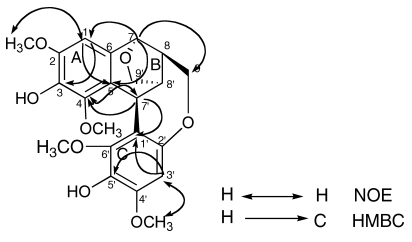



Fig. 2. Key HMBC and NOE Correlations for Determination of the Ovafolinin A (**1**)

and H-8, H-8 and H-8′, and H-7′ and H-8′ (Fig. 2), the relative stereochemistry was assigned to be 7*R**, 8*S**, 7′*R**, and 8′*R**. Accordingly, the structure of ovafolinin A (**1**) was determined as shown in Fig. 1. This assignment was confirmed by single-crystal X-ray diffraction analysis with a suitable single crystal, obtained by careful crystallization from methanol/water (Fig. 3).

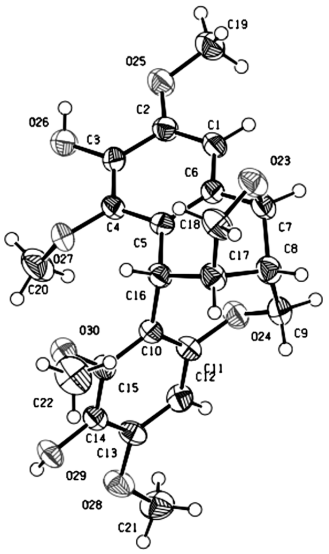



Fig. 3. X-Ray Crystallographic Structure of Ovafolinin A (**1**)

Ovafolinin B (**2**) was obtained as a colorless prism. Its molecular formula was determined to be $C_{22}H_{26}O_8$ by HR-ESI-MS $[M+H]^+$ *m/z* 419.1673 (Calcd for $C_{22}H_{27}O_8$, 419.1706). The IR and UV spectra of **2** were similar to those of **1**. The $^1$H- and $^{13}$C-NMR spectra of **2** were also similar to those of **1**, indicating the presence of four methoxyl groups ($\delta_H$ 3.42, $\delta_C$ 59.6; $\delta_H$ 3.74, $\delta_C$ 55.9; $\delta_H$ 3.81, $\delta_C$ 55.7; $\delta_H$ 4.06, $\delta_C$ 61.5), two aromatic protons ($\delta_H$ 6.25, $\delta_C$ 101.1; $\delta_H$ 6.38, $\delta_C$ 105.4), and one oxymethylene carbon ($\delta_H$ 3.82 and 4.41, $\delta_C$ 80.2) (Tables 1, 2). The similarity in $^1$H–$^1$H COSY and HMBC spectra between **2** and **1** suggest that **2** has the same basic structure as **1**. Although the HMBC correlations between H-7 and C-1, C-8 and C-9 and between H-7′ and C-4, C-5, C-6, C-1′, and C-2′ showed that **2** is aryltetralin type lignan, similar to **1**, a major difference between **2** and **1** was

observed based on the $^{1}$H- and $^{13}$C-NMR chemical shifts at position 7. The proton chemical shifts were $\delta$ 4.75 for **1** and $\delta$ 2.86 and 3.03 for **2** and the carbon chemical shifts were $\delta$ 79.0 for **1** and $\delta$ 29.3 for **2**. This suggests that the oxymethin of **1** is replaced by a methylene in **2**. The NOE correlations between H-1 and $H_a$-7, $H_a$-7 and H-8, and H-7′ and H-8′ of **2** were similar to those of **1** (Fig. 4). Thus, the structure of **2** was determined as shown in Fig. 1.

Ovafolinin C (**3**) was obtained as a yellowish-white amorphous solid. Its molecular formula was determined to be $C_{22}H_{24}O_9$ by HR-ESI-MS $[M+H]^+$ *m/z* 433.1465 (Calcd for $C_{22}H_{25}O_9$, 433.1499). The IR spectrum of **3** indicated the presence of hydroxyl (3272 cm$^{-1}$) and ketone (1670 cm$^{-1}$) groups. The UV absorption maxima occurred at 243 and 290 nm, suggesting the presence of conjugated double bonds in the molecule. The $^{1}$H- and $^{13}$C-NMR spectra of **3** were similar to those of **2**, with the exception that there were only signals for position 7. The signals at $\delta$ 2.86 and 3.03 in **2** were not detected in of the spectra **3**. Furthermore the chemical shift for C-7 of **3** was $\delta$ 200.4 (in $CD_3OD$), whereas that of **2** was $\delta$ 29.3 (in $CDCl_3$). The HMBC correlations observed of H-1, H-8′ and H-8 with C-7, and the IR spectrum indicate the presence of a ketone group. Thus, ovafolinin C (**3**) was assigned as shown in Fig. 1.

Ovafolinin D (**4**) was obtained as a yellowish-white amorphous solid. Its molecular formula was determined to be $C_{22}H_{24}O_8$ by HR-ESI-MS $[M+H]^+$ *m/z* 417.1582 (Calcd for $C_{22}H_{25}O_8$, 417.1549). The IR spectrum showed the presence of hydroxyl groups (3270 cm$^{-1}$). Conjugated systems were also suggested by the UV absorption maximum at 276 nm (log $\varepsilon$ 3.07). The $^{1}$H- and $^{13}$C-NMR spectra of **4** were very similar to those of **1**, indicating the aryltetralin-lignan structure. The $^{1}$H- and $^{13}$C-NMR chemical shifts at position 7 ($\delta$ 4.82, 81.2) at position 9′ ($\delta$ 3.86/3.99, 64.1) and the HMBC correlations of H-7 with C-9′ and of H-9′ with C-7, indicated an ether linkage was assigned between C-7 and C-9′, similar to ovafolinin A (**1**). Furthermore, the HMBC correlations of H-9, H-2′, H-6′ and H-8 with the quaternary carbon C-7′ ($\delta$ 86.0) and the chemical shift of C-9 at $\delta$ 64.1 and of C-7′ at $\delta$ 86.0 demonstrated the presence of an ether linkage between C-9 and C-7′. The absolute configuration of ovafolinin D (**4**) was established by comparison of ovafolinin D dimethyl ether (**9**) from ovafolinin D (**4**) and that prepared from lyoniside (**6**) as shown in Chart 1. Because the two derivatives were indistinguishable in the NMR and MS data, the absolute structure of **4** was determined as shown in Fig. 1.

Ovafolinin E (**5**) was obtained as a yellowish-white amorphous solid. Its molecular formula was determined to be $C_{22}H_{24}O_8$ by HR-ESI-MS $[M+H]^+$ *m/z* 417.1562 (Calcd for $C_{22}H_{25}O_8$ 417.1549). The IR spectrum showed absorptions for hydroxyl (3306 cm$^{-1}$) and carbonyl groups (1660 cm$^{-1}$). The UV absorption maxima occurred at 253 (log $\varepsilon$ 4.10) and 354 nm (log $\varepsilon$ 4.01), implying the presence of conjugated double bond systems in the molecule. The $^{1}$H- and $^{13}$C-NMR spectra showed the signals attributed to an aldehyde group ($\delta$ 9.49, 194.3) and olefinic carbons ($\delta$ 149.0, 136.4). The aldehyde group was located at position 9 based on correlations between H-7 and C-9, H-9 and C-8 ($\delta$ 136.3) in HMBC spectrum data. Analysis of the $^{1}$H–$^{1}$H COSY, HMQC, and HMBC spectra, ovafolinin E (**5**), unlike the others (**1**—**4**), has no ether linkage between any of carbons in the aryltetralin-lignan skeleton (Fig. 1).

Ovafolinins A (**1**)—E (**5**) were found to be inactive in the cytotoxic activity test on HL 60, HCT116, A549, and MCF7 cell lines.

Ovafolinins A (**1**)—C (**3**) were determined to be unique

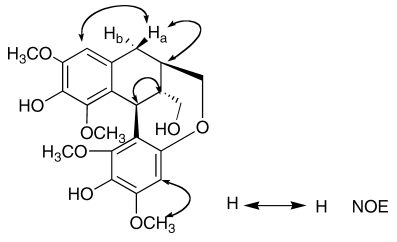
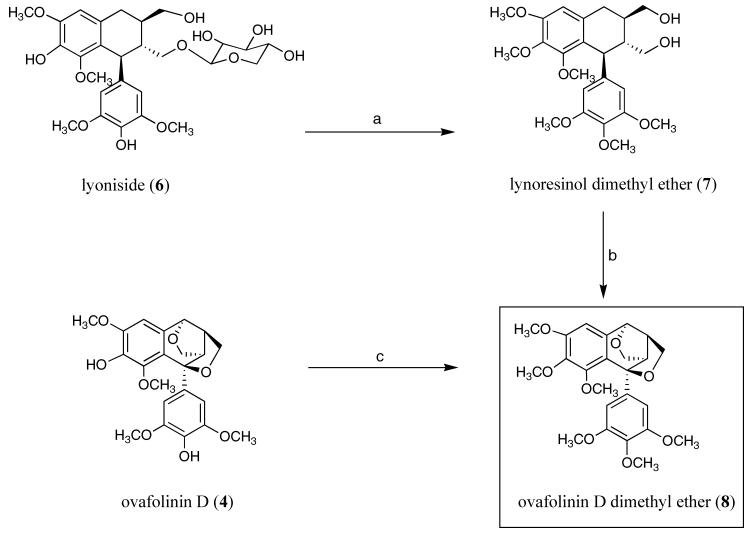



Fig. 4. Selected NOE Correlations of Ovafolinin B (**2**)




Reagent; (a) $CH_3I/K_2CO_3$, aceton, 5%$H_2SO_4$, (b) $CuSO_4/K_2S_2O_8$, $CH_3CN$, (c) $CH_3I/K_2CO_4$.

Chart 1. Derivation of Ovafolinin D Dimethyl Ether (**8**) from Lyoniside (**6**) and Ovafolinin D (**4**)

aryltetralin-lignans containing 7-membered ring formed by the C9–C-2′ ether linkage. **1**—**3** bear a benzoxepin structures including the C ring of the aryltetralin-lingnan. In addition, ovafolinin D (**4**) is the first example of a naturally occurring aryltetralin-lignan bearing methyleneoxy-bridge.

## Experimental

**General Procedures** Optical rotations were measured on a JASCO DIP-1000 digital polarimeter. IR spectra were recorded on a FT-IR 1710 spectrophotometer. UV spectra were obtained using a Hitachi U-2001 spectrophotometer. NMR spectra were measured on Bruker DRX-500 and DPX-400 spectrometers at 300 K. The $^1$H-NMR chemical shifts in $CDCl_3$ and $CD_3OD$ calibrated to the residual $CHCl_3$ and $CH_3OH$ resonances at 7.26 and 3.31 ppm, respectively, and the $^{13}$C-NMR chemical shifts were calibrated to the solvent peaks at 77.0 and 49.0 ppm, respectively. Mass spectra were obtained using a Micromass LCT spectrometer. Preparative HPLC was carried out on a Shimadzu LC-6AT system equipped with a SPD-10AVP detector and a reversed-phased column, Mightysil RP-18 prep (5 $\mu$g, 20×250 mm), using $CH_3OH$ : $H_2O$ or a $CH_3CN$ : $H_2O$ as the mobile phase, at a flow rate of 5 ml/min.

**Plant Collection** Wood from *L. ovalifolia* was collected in Saitama Prefecture, in October 2000, and the plant origin was identified by Dr. H. Ina (Tokyo University of Pharmacy and Life Sciences, Japan).

**Extraction and Isolation** Fresh wood chips (15 kg) from *L. ovalifolia* were extracted using MeOH. After removal of MeOH *in vacuo*, the residue was dissolved in water. The aqueous layer was separated from the precipitates, and placed on a HP-20 column (DIAION). Elution with $H_2O$/MeOH mixtures (100 : 0, 80 : 20, 60 : 40, 40 : 60, 20 : 80 and 0 : 100) and acetone afforded seven fractions (frs. Lo 1—7). Fraction Lo 5 (26.1 g) was subjected to silica gel (Merck Kieselgel 60) column chromatography eluting sequentially with $CHCl_3$/MeOH mixture (50 : 1, 20 : 1, 10 : 1, 7 : 3, and 1 : 1). The $CHCl_3$/$CH_3OH$ (50 : 1) fraction (681.7 mg) was evaporated and applied to ODS HPLC eluting with $CH_3CN$ : $H_2O$ (60 : 40) to afford **1** (37.8 mg) and **5** (3.6 mg), and with $CH_3CN$ : $H_2O$ (70 : 39) to afford **2** (5.2 mg), **3** (3.2 mg) and **4** (3.7 mg).

Ovafolinin A (**1**): Colorless prisms, $[\alpha]_D^{25}$ −37.3° ($c$=0.36, MeOH). IR (neat) cm$^{-1}$: 3392, 2940, 1615. UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 243 (3.86), 283 (3.58). $^1$H- and $^{13}$C-NMR spectrometric data are given in Tables 1 and 2, respectively. HR-ESI-MS $m/z$: 417.1523 $[M+H]^+$ (Calcd for $C_{22}H_{25}O_8$, 417.1549).

Ovafolinin B (**2**): Colorless prisms, $[\alpha]_D^{25}$ +52.0° ($c$=0.26, MeOH). IR (neat) cm$^{-1}$: 3370, 2936, 1613. UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 283 (3.99) nm. $^1$H- and $^{13}$C-NMR spectrometric data are given in Tables 1 and 2, respectively. HR-ESI-MS $m/z$: 419.1673 $[M+H]^+$ (Calcd for $C_{22}H_{27}O_8$ 419.1706).

Ovafolinin C (**3**): Amorphous solid, $[\alpha]_D^{25}$ +105.7° ($c$=0.11, MeOH). IR (neat) cm$^{-1}$: 3272, 2938, 1567. UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 290 (3.85). $^1$H- and $^{13}$C-NMR spectrometric data are given in Tables 1 and 2, respectively. HR-ESI-MS $m/z$: 433.1465 $[M+H]^+$ (Calcd for $C_{22}H_{25}O_9$ 433.1499).

Ovafolinin D (**4**): Amorphous solid, $[\alpha]_D^{25}$ −33° ($c$=0.09, MeOH). IR (neat) cm$^{-1}$: 3389, 2930, 1614. UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 276 (3.07). $^1$H- and $^{13}$C-NMR spectrometric data are given in Tables 1 and 2, respectively. HR-ESI-MS $m/z$: 417.1582 ($[M+H]^+$ (Calcd for $C_{22}H_{25}O_8$ 417.1549).

Ovafolinin E (**5**): Amorphous solid. $[\alpha]_D^{25}$ +105.7° ($c$=0.16, MeOH); IR (neat) cm$^{-1}$: 3272, 2938, 1567. UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 290 (3.85). $^1$H- and $^{13}$C-NMR spectrometric data are given in Tables 1 and 2, respectively. HR-ESI-MS $m/z$: 417.1562 $[M+H]^+$ (Calcd for $C_{22}H_{25}O_8$ 417.1549).

**Conversion of Lyoniside (6) to Lyoniresinol Dimethyl Ether (8)** Lyoniside (**6**, 1 g, 1.8 mmol) and $CH_3I$ (1 ml) were stirred in acetone (40 ml) at room temperature for 66 h to afford lyoniside dimethyl ether (330 mg) by silica gel chromatography ($CHCl_3$ : MeOH=10 : 1). This product was dissolved in EtOH (10 ml) and treated with an aqueous solution 5% $H_2SO_4$ (2 ml) at 110 °C under reflux for 7 h. After cooling, $H_2O$ was added to the mixture, and it was extracted with AcOEt. The AcOEt layer was dried over $Mg_2SO_4$, evaporated, and subjected to silica gel column chromatography ($CHCl_3$ : MeOH=20 : 1). The product was isolated and crystallized from benzene to yield lyoniresinol dimethyl ether (**8**, 208 mg). Lyoniresinol dimethyl ether: HR-ESI-MS $[M+Na]^+$ $m/z$: 471.2015 (Calcd for $C_{24}H_{32}O_8Na$, 471.2019).

**Oxidation of Lyoniresinol Dimethyl Ether (8)**[12,13] Lyoniresinol dimethyl ether (**8**, 40 mg, 0.089 mmol, 14.4 ml $CH_3CN$), $CuSO_4 \cdot 5H_2O$ (24 mg, 0.15 mmol, 1.6 ml $H_2O$) and $K_2S_2O_8$ (51.6 mg, 0.19 mmol, 4.4 ml $H_2O$) were refluxed for 0.5 h at 120 °C, diluted with $H_2O$, and extracted with AcOEt. Ovafolinin D dimethyl ether (**9**, 2.9 mg) was isolated by HPLC ($CHCl_3$ : AcOEt=3 : 1). Ovalifolin D dimethyl ether (**9**); Amorphous solid, $[\alpha]_D^{25}$ −60° ($c$=0.05, MeOH). $^1$H-NMR ($CDCl_3$) $\delta$: 7.07 (1H, d, $J$=1.7 Hz, H-2′), 6.61 (1H, s, H-6), 6.39 (1H, d, $J$=1.7 Hz, H-6′), 4.83 (1H, d, $J$=5.4 Hz, H-7), 4.25 (1H, dd, $J$=6.4, 9.5 Hz, H-9a), 4.04 (1H, dd, $J$=6.3, 9.8 Hz, H-9′a), 3.93 (1H, overlapped, H-9′b), 3.92 (3H, s, H-3′), 3.90 (3H, s, H-3), 3.86 (3H, s, H-4′), 3.76 (3H, s, H-4), 3.73 (3H, s, H-3′), 3.46 (1H, dd, $J$=8.5, 9.5 Hz, H-9b), 3.32 (3H, s, H-5′), 3.13 (1H, dd, $J$=6.4, 9.5 Hz, H-8), 2.73 (1H, dd, $J$=6.3, 6.1 Hz, H-8′). $^{13}$C-NMR ($CDCl_3$) $\delta$: 154.0 (C-3), 153.2 (C-3′), 152.5 (C-5′), 152.4 (C-5), 143.4 (C-4), 139.7 (C-1′), 136.4 (C-4′), 131.0 (C-1), 121.1 (C-2), 106.7 (C-6), 101.2 (C-6′), 100.2 (C-2′), 85.9 (C-7′), 81.0 (C-7), 64.3 (C-9), 64.0 (C-9′), 60.8 (–$OCH_3$), 60.6 (–$OCH_3$×2), 57.9 (C-8′), 56.1 (–$OCH_3$), 56.0 (–$OCH_3$), 55.9 (–$OCH_3$), 49.0 (C-8). HR-ESI-MS $m/z$: 445.1860 $[M+H]^+$ (Calcd for $C_{24}H_{29}O_8Na$, 445.1862).

**Methylation of Ovafolinin D (4)** $CH_3I$ (1 $\mu$l) and anhydrous $K_2CO_3$ (8 mg) were added to a solution **4** (1.2 mg, 0.0029 mmol) in acetone (0.04 ml) while stirring. Then the mixture maintained with stirring for 30 h at room temperature. After evaporation and purification of the crude product by HPLC ($CHCl_3$ : AcOEt=3 : 1) afforded the methylated product (0.5 mg). $^1$H-NMR ($CDCl_3$) $\delta$: 7.08 (1H, d, $J$=1.7 Hz, H-2′), 6.62 (1H, s, H-6), 6.39 (1H, d, $J$=1.7 Hz, H-6′), 4.82 (1H, d, $J$=5.3 Hz, H-7), 4.26 (1H, dd, $J$=6.3, 9.7 Hz, H-9a), 4.04 (1H, dd, $J$=6.3, 9.7 Hz, H-9′a), 3.93 (1H, overlapped, H-9′b), 3.93 (3H, s, H-3′), 3.90 (3H, s, H-3), 3.86 (3H, s, H-4′), 3.74 (3H, s, H-4), 3.73 (3H, s, H-3′), 3.46 (1H, overlapped, H-9b), 3.33 (3H, s, H-5′), 3.12 (1H, dd, $J$=6.3, 9.5 Hz, H-8), 2.73 (1H, dd, $J$=6.3, 6.1 Hz, H-8′). HR-ESI-MS $m/z$: 445.1882 $[M+H]^+$ (Calcd for $C_{24}H_{29}O_8Na$, 445.1862).

**X-Ray Crystallographic Study of Ovalifolin A (1)** $C_{22}H_{24}O_8$, M=416.41, 0.49×0.45×0.38. Single-crystal X-ray analysis was carried out on a Mac Science DIP diffractometer with Mo$K\alpha$ radiation ($\lambda$=0.71073). The data indicated the monoclinic space group, $Cc$, $a$=19.6130 (19) Å, $b$=7.0010 (7) Å, $c$=15.2280 (8) Å, $V$=1955.5 (3) Å$^3$, $Z$=4, $D_x$=1.414 Mg m$^{-3}$, 2081 measured reflections, 2081 independent reflections, 1589 observed reflections [$I>2\sigma(I)$], $R1$=0.0387, $wR2$=0.0899 (observed data), GOF=0.930; $R1$=0.0476, $wR2$=0.0925 (all data). The structure was solved by direct methods using the maXus crystallographic software package, and refined by full-matrix least-squares on F2 using the program SHELXL-97. Crystallographic data for the structure of **1** have been deposited in the Cambridge Crystallographic Data Centre under deposition number CCDC 722340.

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