# Phenolic Compounds of *Isodon oresbius*

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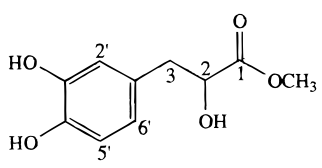
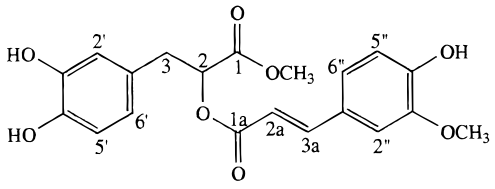
Two new compounds, oresbiusin A (**1**) and B (**2**), have been isolated from *Isodon oresbius*. The structures of **1** and **2** were elucidated as methyl 3-(3′,4′-dihydroxyphenyl) lactate and methyl 2-*O*-feruloyl-3-(3′,4′-dihydroxyphenyl) lactate, respectively, on the basis of spectroscopic evidence.

The whole herb of *Isodom oresbius* (W. W. Smith) Kudo (Labiatae) has been used in Chinese traditional medicine for the treatment of blood clots in internal organs of the body.[1] The species is native to Sichuan and Yunnan, China, and has not been previously investigated chemically. Two new phenolic compounds **1** and **2**, along with ferulic acid, *p*-hydroxycinnamic acid, rosmarinic acid, and methyl rosmarinate, were isolated from the whole plant. This paper deals with the isolation and structural elucidation of **1** and **2**.




**1**




**2**

The AcOEt-soluble portion of the EtOH extract of *I. oresbius* was subjected to repeated chromatography to afford oresbiusin A (**1**) and oresbiusin B (**2**) and four known compounds, ferulic acid, *p*-hydroxycinnamic acid, rosmarinic acid, and methyl rosmarinate. The structures of **1** and **2** were elucidated by spectroscopic methods (in particular 1D and 2D NMR). The known compounds were identified by comparing their mp's, MS, $^1$H NMR, and $^{13}$C NMR data with those described in the literature.[2−5]

Compound **1** was obtained as a light yellow amorphous solid, $C_{10}H_{12}O_5$ (FABMS). Its IR spectrum showed the presence of hydroxy (3500 cm$^{-1}$), aromatic (1600, 1530, and 1450 cm$^{-1}$) and carbonyl (1720 cm$^{-1}$) groups. The $^{13}$C NMR spectrum of **1** exhibited, in addition to the signal of methoxy group ($\delta$ 51.5), nine carbons including six aromatic carbons ($\delta$ 129.7 s, 118.1 d, 147.0 s, 145.9 s, 116.4 d, and 121.1 d), one methylene ($\delta$ 41.1 t), one carbinol ($\delta$ 73.1 d), and one carbonyl carbon ($\delta$ 175.2 s). Taking into account the above data for a total of five degrees of unsaturation, **1** was assumed to possess a phenyl propanoid skeleton.

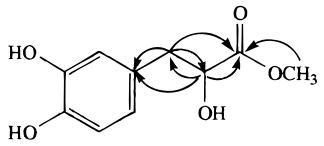



**Figure 1.** HMBC spectrum of **1** (correlations observed in benzene ring were omitted).

The $^1$H NMR spectrum of **1** revealed signals attributable to a methoxy group ($\delta$ 3.60 s), aliphatic ABX-spin pattern protons at $\delta$ 4.74 (dd, $J$ = 7.6, 5.0 Hz), 3.30 (dd, $J$ = 13.7, 5.0 Hz), and 3.15 (dd, $J$ = 13.7, 7.6 Hz) and aromatic ABX-type protons at $\delta$ 7.16 (d, $J$ = 8 Hz), 6.91 (dd, $J$ = 8.0, 2.0 Hz), and 7.40 (br s), typical of an unsymmetrically trisubstituted benzene ring.

The EIMS of **1** furnished a characteristic strong peak at $m/z$ 163 corresponding to the caffeoyl cation, thus placing the methoxyl group on C-1 rather than on the benzene ring and indicating that **1** was a derivative of *o*-dihydroxyphenyl lactic acid.[5] Attachment of the methoxy group to C-1 was further confirmed by the correlation between HOMe and C-1 in the HMBC spectrum (Figure 1). Thus, it was deduced that **1** is methyl 3-(3′,4′-dihydroxyphenyl) lactate.

Compound **2**, a yellow amorphous solid, gave rise to [M]$^+$ at $m/z$ 388 (EIMS) in agreement with the molecular formula $C_{20}H_{20}O_7$. The presence of hydroxy (3300 cm$^{-1}$), carbonyl (1700 cm$^{-1}$), and an aromatic (1600, 1500 cm$^{-1}$) group was indicated by the IR spectrum. The $^1$H NMR spectrum of **2** exhibited two sets of aromatic ABX-type signals at $\delta$ 7.51 (d, $J$ = 2 Hz), 7.22 (d, $J$ = 8 Hz), 6.88 (d, $J$ = 8 Hz), and at $\delta$ 7.38 (d, $J$ = 2 Hz), 7.05 (d, $J$ = 8 Hz), 6.91 (d, $J$ = 8 Hz); aliphatic ABX-type signals at $\delta$ 5.70 (dd, $J$ = 7.3, 5.0 Hz), 3.62 (dd, $J$ = 13.7, 5.0 Hz), and 3.30 (dd, $J$ = 13.7, 7.3 Hz). An AB system [$\delta$ 7.96 (d, $J$ = 15.9 Hz), 6.66 (d, $J$ = 15.9 Hz)] was assigned to *trans*-olefinic protons. Additionally, two singlets at $\delta$ 3.60 (3H, s) and 3.70 (3H, s) were ascribable to two methoxy groups. These findings suggested that **2** was composed of two phenyl propanoid subunits.

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Subunit **a** was immediately appearent from its striking similarity of $^1$H NMR signals with those of **1**.

From the base peak at *m/z* 194, in the MS of **2**, it was appearent that the molecular ion readily gave two fragments of equal mass by a McLafferty rearrangement. Subunit **b** was then judged to be a *trans*-feruloyl or *trans*-isoferuloyl group.

To determine the substitution pattern of the benzene ring for subunit **b**, a NOE experiment was carried out. Irradiation of the methoxyl signal ($\delta$ 3.70) caused a 20% increase in the integrated intensity of the C-2″ proton signal ($\delta$ 7.51, d, $J$ = 2.0 Hz). Thus, subunit **b** was concluded to be a *trans*-feruloyl group.

Analysis of the $^{13}$C NMR spectrum of **2** confirmed the presence of subunits **a** and **b** and demonstrated conclusively that subunit **b** was attached to the C-2 oxygen of subunit **a** upon the following evidence.

Comparison of the $^{13}$C NMR spectra of **1** and **2** revealed that the signals due to C-1, C-3, and C-2 differed by 4.2, 3.6, and 0.9 ppm, respectively, typically observed on esterification.[7] The structure of **2** was thus revealed to be methyl 2-*O*-feruloyl-3-(3′,4′-dihydroxyphenyl) lactate.

## Experimental Section

**General Experimental Procedures.** Melting points were determined using a Kofler hot-stage apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 567 spectrophotometer. $^1$H and $^{13}$C NMR spectra were recorded on a Bruker AM-400 instrument using TMS as internal standard. MS was obtained using a DX-300 instrument. TLC column chromatography were performed on Si gel.

**Plant Material.** The whole herbs of *I. Oresbius* were collected from Lijiang County, Yunnan Province, China, in Aug 1993 and identified by Prof. H.-W. Li. A voucher specimen was deposited in the Herbarium of the Department of Taxonomy, Kunming Institute of Botany, Academia Sinica, Kunming, China.

**Extraction and Isolation.** The powdered, air-dried whole herbs of *I. oresbius* (2.8 kg) were extracted exhaustively with hot 95% EtOH (5 L), and the solution was concentrated under reduced pressure to give a dark green residue (600 g). This residue was redissolved in 600 mL of boiling $H_2O$ and filtered, and the aqueous fitrate was partitioned with hexane, EtOAc, and *n*-BuOH.

The EtOAc residue (140 g) was subjected to column chromatography (Si gel), eluting with $CHCl_3$ and increasing proportions of MeOH−$CHCl_3$. Fractions were monitored by TLC. All compounds were further purified by recrystallization and PTLC (Si gel), yielding the compounds in order of increasing polarity: ferulic acid (25 mg), *p*-hydroxycinnamic acid (18 mg), rosmarinic acid (80 mg), methyl rosmarinate (50.2 mg), oresbiusin A (**1**) (20.0 mg), and oresbiusin B (**2**) (18.8 mg).

**Oresbiusin A (1).** Compound **1** was obtained as a light yellow amorphous solid (20.0mg) that could not be induced to crystallize: IR (KBr) $\nu_{max}$ 3500−3200, 1720, 1600, 1510, 1450 cm$^{-1}$; $^1$H NMR ($C_5D_5N$, 400.13 MHz) $\delta$ 7.40 (1H, br s, H-2′), 7.16 (1H, d, $J$ = 8.0 Hz, H-5′), 6.91 (1H, dd, $J$ = 8.0, 2.0 Hz, H-6′), 4.74 (1H, dd, $J$ = 7.6, 5.0 Hz, H-2), 3.60 (3H, s, OMe), 3.30 (1H, dd, $J$ = 13.7, 5.0 Hz, H-3α), 3.15 (1H, dd, $J$ = 13.7, 7.6 Hz, H-3$\beta$); $^{13}$C NMR ($C_5D_5N$, 100.62 MHz) $\delta$ 175.2 (s, C-1), 147.0 (s, C-3′), 145.9 (s, C-4′), 129.7 (s, C-1′), 121.1 (d, C-6′), 118.1 (d, C-2′), 116.4 (d, C-5′), 73.1 (d, C-2), 51.5 (s, Me), 41.1 (t, C-3); EIMS *m/z* 212 ($M^+$, 80), 194 (70), 163 (78), 152 (60), 136 (60), 125 (75), 110 (80), 95 (60), 78 (100); FABMS *m/z* 211 $[M - H]^-$.

**Oresbiusin B (2).** Compound **2** was isolated as a yellowish viscous solid (18.8 mg) that also could not be induced to crystallize: TLC was performed on Si gel with (A) $CHCl_3$−MeOH−AcOH (90:10:1), $R_f$ 0.35, (B) $CHCl_3$−MeOH−$H_2O$ (65:35:10, organic phase) $R_f$ 0.30; IR $\nu_{max}$ 3500−3200, 1700, 1620, 1600, 1500, 1450 cm$^{-1}$; $^1$H NMR ($C_5D_5N$, 400.13 MHz) $\delta$ 7.96 (1H, d, $J$ = 15.9 Hz, H-3a), 7.51 (1H, d, $J$ = 2.0 Hz, H-2″), 7.38 (1H, d, $J$ = 2.0 Hz, H-2′), 7.22 (1H, d, $J$ = 8.0 Hz, H-5″), 7.05 (1H, d, $J$ = 8.0 Hz, H-5′), 6.91 (1H, d, $J$ = 8.0 Hz, H-6′), 6.88 (1H, d, $J$ = 8.0 Hz, H-6″), 6.66 (1H, d, $J$ = 15.9 Hz, H-2a), 5.70 (1H, dd, $J$ = 7.3, 5.0 Hz, H-2), 3.70 (3H, s, OMe-3″), 3.62 (1H, dd, $J$ = 13.7, 5.0 Hz, H-3α), 3.60 (3H, s, OMe-1), 3.30 (1H, dd, $J$ = 13.7, 7.3 Hz, H-3$\beta$); $^{13}$C NMR ($C_5D_5N$, 100.62 MHz) $\delta$ 171.0 (s, C-1), 166.0 (s, C-1a), 151.2 (s, C-3″), 148.5 (s, C-4″), 147.3 (d, C-3a), 146.6 (s, C-3′), 146.4 (s, C-4′), 128.0 (s, C-1″), 128.0 (s, C-1′), 121.1 (d, C-6″), 119.2 (d, C-6′), 117.2 (d, C-5′), 116.4 (s, C-5″), 115.8 (d, C-2a), 115.3 (d, C-2′), 112.2 (d, C-2″), 74.0 (d, C-2), 55.9 (s, OMe), 55.3 (s, OMe), 37.5 (t, C-3); EIMS *m/z* 388 ($M^+$, 3), 358 (2), 208 (4), 194 (100), 177 (45), 163 (18), 123 (70), 105 (10), 89 (20), 77 (25).

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