

Phenolic Compounds of *Isodon oresbius*

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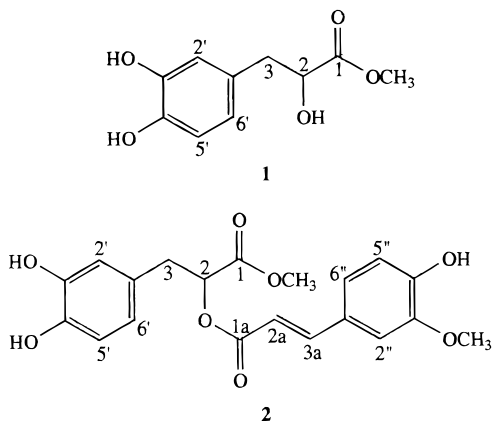
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Two new compounds, oresbiusin A (**1**) and B (**2**), have been isolated from *Isodon oresbius*. The structures of **1** and **2** were elucidated as methyl 3-(3',4'-dihydroxyphenyl) lactate and methyl 2-*O*-feruloyl-3-(3',4'-dihydroxyphenyl) lactate,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

The whole herb of *Isodon oresbius* (W. W. Smith) Kudo (Labiateae) 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**.



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, ¹H NMR, and ¹³C NMR data with those described in the literature.^{2–5}

Compound **1** was obtained as a light yellow amorphous solid, C₁₀H₁₂O₅ (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 ¹³C NMR spectrum of **1** exhibited, in addition to the signal of methoxy group (δ 51.5), nine carbons including six aromatic carbons (δ 129.7 s, 118.1 d, 147.0

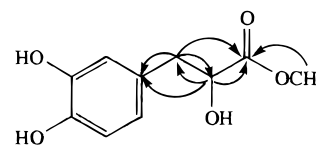


Figure 1. HMBC spectrum of **1** (correlations observed in benzene ring were omitted).

s, 145.9 s, 116.4 d, and 121.1 d), one methylene (δ 41.1 t), one carbinol (δ 73.1 d), and one carbonyl carbon (δ 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.

The ¹H NMR spectrum of **1** revealed signals attributable to a methoxy group (δ 3.60 s), aliphatic ABX-spin pattern protons at δ 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 δ 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 C₂₀H₂₀O₇. 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 ¹H NMR spectrum of **2** exhibited two sets of aromatic ABX-type signals at δ 7.51 (d, *J* = 2 Hz), 7.22 (d, *J* = 8 Hz), 6.88 (d, *J* = 8 Hz), and at δ 7.38 (d, *J* = 2 Hz), 7.05 (d, *J* = 8 Hz), 6.91 (d, *J* = 8 Hz); aliphatic ABX-type signals at δ 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 [δ 7.96 (d, *J* = 15.9 Hz), 6.66 (d, *J* = 15.9 Hz)] was assigned to *trans*-olefinic protons. Additionally, two singlets at δ 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 apparent from its striking similarity of ^1H NMR signals with those of **1**.

From the base peak at m/z 194, in the MS of **2**, it was apparent 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 (δ 3.70) caused a 20% increase in the integrated intensity of the C-2'' proton signal (δ 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. ^1H 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 filtrate 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) ν_{max} 3500–3200, 1720, 1600, 1510, 1450 cm^{-1} ; ^1H NMR ($\text{C}_5\text{D}_5\text{N}$, 400.13 MHz) δ 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-3 α), 3.15 (1H, dd, $J = 13.7, 7.6$ Hz, H-3 β); ^{13}C NMR ($\text{C}_5\text{D}_5\text{N}$, 100.62 MHz) δ 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 [$\text{M} - \text{H}$] $^-$.

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 ν_{max} 3500–3200, 1700, 1620, 1600, 1500, 1450 cm^{-1} ; ^1H NMR ($\text{C}_5\text{D}_5\text{N}$, 400.13 MHz) δ 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-3 α), 3.60 (3H, s, OMe-1), 3.30 (1H, dd, $J = 13.7, 7.3$ Hz, H-3 β); ^{13}C NMR ($\text{C}_5\text{D}_5\text{N}$, 100.62 MHz) δ 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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