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Full Length Research Paper

Isolation, characterization and crystal structure of ethyl 4-hydroxy-3,5-dimethoxy-benzoate from *Scutellaria barbata*

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Phytochemical research on the aerial parts of *Scutellaria barbata* used variously in ethno medicinal treatments in southern China, resulted in the isolation of ethyl 4-hydroxy-3,5-dimethoxy-benzoate. Its structure was identified purely on the basis of spectroscopic analyses including EI-MS, IR and X-ray diffraction analysis. The crystal belonged to the monoclinic space group P2 (1)/c with a = 11.5521(6) Å, b = 13.5055(7) Å, c = 16.4171(7) Å, β = 117.240(3)° and Z = 8.This compound was firstly isolated from the genus *Scutellaria*.

Key words: *Scutellaria barbata*, isolation, x-ray diffraction analysis, ethyl 4-hydroxy-3,5-dimethoxy-benzoate.

INTRODUCTION

As a popular traditional medicinal herb listed in the Chinese Pharmacopoeia, *Scutellaria barbata* D.Don (Lamiaceae) has a therapeutic history extending back over thousands of years and has attracted a great deal of attention as an anticancer agent in Chinese herbal medicine. *S. barbata* is distributed natively throughout Korea and southern China as a perennial herb. In the clinic, the herb has been used in the treatment of digestive system cancers, hepatoma, lung cancer, breast cancer and other diseases (Lee et al., 2004; Goh et al., 2005; Cha et al., 2004; Suh et al., 2007). Chemical constituents isolated from the plant including flavonoids (Sato et al., 2000), neo-clerodane diterpenoids (Zhu et al., 2009, 2010; Lee et al., 2010; Wang et al., 2010) and alkaloids (Dai et al., 2007, 2008a, b, 2009a, b), polysaccharides, volatile oils (Yu et al., 2004) and so on. In recent years, more and more compounds have been isolated from the plant. However, numerous compounds of this herb are still unknown. In the course of our seeking for more new constituents, we started phytochemical research on the air-dried aerial parts of *S. barbata* and isolated of ethyl 4-hydroxy-3,5-dimethoxy-benzoate. In the present paper, we would like to describe the isolation and structure elucidation of the compound from *S. barbata*.

MATERIALS AND METHODS

General

Melting point was determined on a MPA100 (OptiMelt) automated melting point system. The UV spectrum was recorded using HP-8453 spectrophotometer in the wavelength range 200 to 500 nm. EI-MS data were obtained with a DSQ MS spectrometer. IR spectrum was recorded with VECTOR 22 infrared spectrometer using KBr pellet.X-ray single-crystal diffraction measurement was carried out at 298(2) K on a Bruker Smart 1000 CCD area diffractometer equipped with graphite monochromatic MoKa radiation (k = 0.71073 Å) for data collection. The final refinement was performed by full-matrix least squares techniques with anisotropic thermal parameters for the non-hydrogen atoms on F2.

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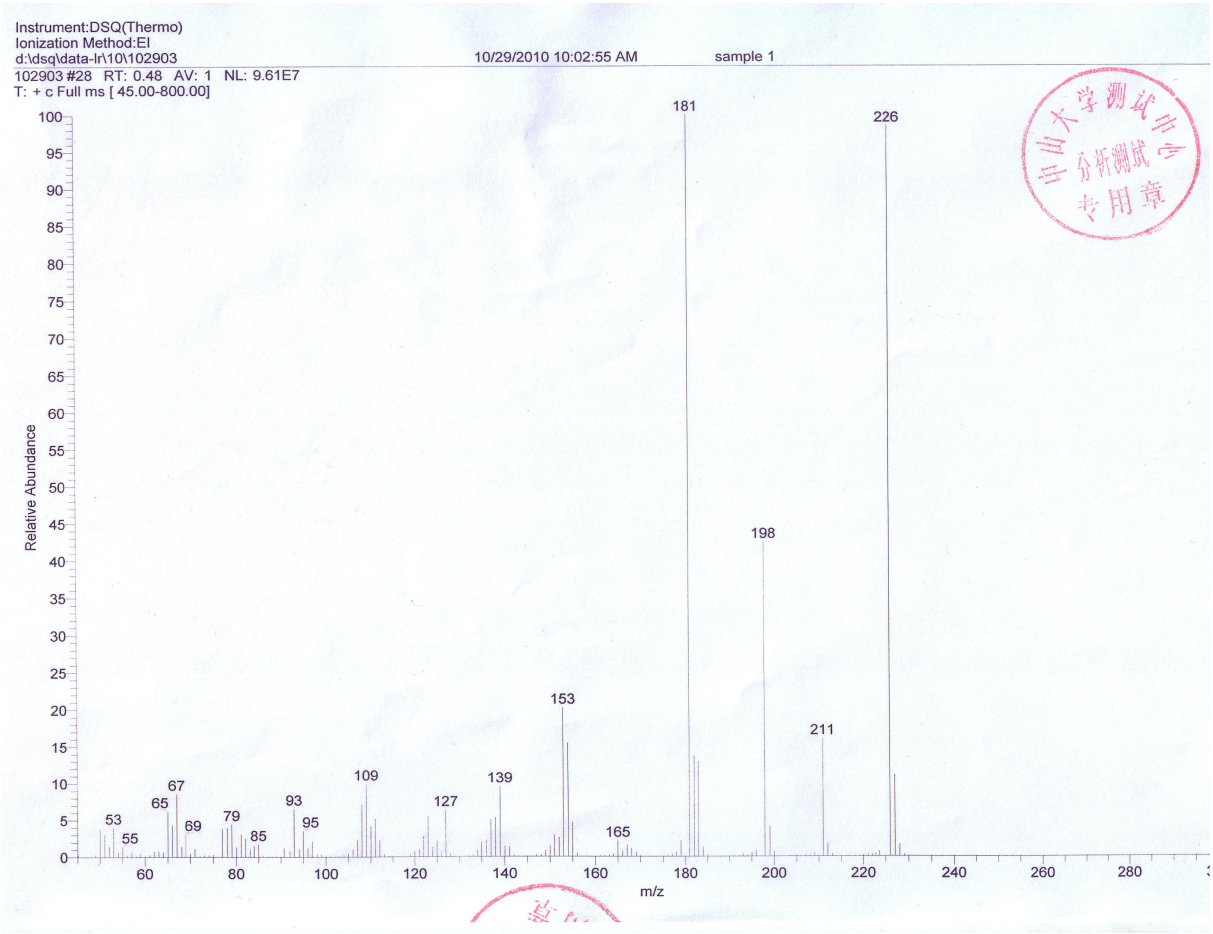

Figure 1. The EI-MS of the compound.

All the H atoms were placed in the calculated positions and constrained to ride on their parent atoms. Column chromatography was performed by using silica gel (200 to 300 mesh, Qingdao Marine Chemical Factory, People's Republic of China) and analytical TLC was performed on precoated plates and visualized under UV_{366} light.

Plant material

S. barbata was collected in Queshan County, Henan Province, China, in September 2009, and identified by Prof. Shouyao Zhang, Department of Pharmacy, Zhujiang Hospital, Southern Medical University, Guangdong, China. A voucher specimen, number 090912, was deposited there. The collected sample was air-dried, pulverized using a mill hammer and stored in polythene bags for use.

Extraction and Isolation

The air-dried aerial parts of *Scutellaria barbata* (25.6 kg) was finely cut and soaked in acid ethanol (12 mol/L HCl-95% EtOH, 2:100, v/v) for 72 h at room temperature, then extracted three times(1 h x 3) in an ultrasonic bath with acid ethanol (40 l x 3). After evaporating of the solvent under reduced pressure, the extract (2.15 kg) was dissolved and suspended in 2% HCl (8.0 l), stood overnight and filtrated. The acidic solution was basified to pH 10 with NH_4OH (25%) and exhaustively extracted with $CHCl_3$ (6 l x 5).The organic fractions were combined and evaporated under vacuum to yield the alkaloidal fraction (19.8 g). The alkaloidal fraction was initially subjected to column (3.0 x 80 cm) chromatography on silica gel (200 to 300 mesh, 160 g), eluted with petroleum ether-acetone [95:5 (3.0 l), 90:10 (5.0 l), 85:15 (5.0 l), 80:20 (7.0 l), 75:25 (7.0 l), 70:30 (7.0 l), 60:40 (5.0 l), 50:50 (5.0 l), 40:60 (5.0 l)] to give nine fractions. Fraction 4 was separated by silica gel column (2.0 x 80 cm, 200 to 300 mesh, 60 g) chromatography, eluted by petroleum ether-acetone (95:10, v/v), and then yielded crude crystalline solid which afforded a white granular crystal (35.8 mg) after recrystallization with petroleum ether–acetone (9:1, v/v).

RESULTS AND DISCUSSION

A acidic ethanol extract of air-dried aerial parts of *S. barbata* was processed as described in the experimental section to provide a compound, which was isolated as a white granular crystal in a 0.00014% yield based on the dry material. By the combined analysis of EI-MS which showed a molecular ion at 226 [M^+] (Figure 1) and X-ray diffraction analysis, the molecular formula was proposed

Table 1. Crystal data and structure refinement for the compound.

Crystal data	
Empirical formula	C_{11} H_{14} O_5
Formula weight	226.22
Temperature (K)	293(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
space group	P2(1)/c
a (Å)	11.5521(6)
b (Å)	13.5055(7)
c (Å)	16.4171(7)
a (°)	90
b (°)	117.240(3)
g (°)	90
Volume (Å3)	2277.28(19)
Z	8
Calculated density	1.320 Mg/m^3
Absorption coefficient	0.105 mm^{-1}
F(0 0 0)	960
Crystal size	0.45 x 0.44 x 0.42 mm^3
Theta range for data collection (°)	1.98 to 26.96
Limiting indices	-14≤h≤14, -17≤k≤16, -20≤l≤20
Reflections collected/unique	14358
Completeness to θ = 26.96°	98.1%
Independent reflections	4857 [R(int) = 0.0281]
Refinement method	Full-matrix least-squares on F2
Data/restraints/parameters	4857 / 0 / 297
Goodness-of-fit on F2	1.003
Final R indices [I > 2σ(I)]	R1 = 0.0454, wR2 = 0.1229
R indices (all data)	R1 = 0.0764, wR2 = 0.1445
Largest diff. peak and hole (eÅ-3)	0.161 and -0.203

Table 2. Hydrogen-bonding geometry for the compound.

D-H...A	D-H (Å)	H...A (Å)	D...A (Å)	D-H...A (°)
O(4)-H(4)...O(1)#1	0.82	2.08	2.7826(17)	143.4
O(9)-H(9)...O(6)#2	0.82	2.08	2.8553(18)	157.3

Symmetry transformations used to generate equivalent atoms: #1 -x+1,y+1/2,-z+1/2, #2 -x,y+1/2,-z+1/2.

as $C_{11}H_{14}0_5$. Suitable crystal of the compound for X-ray diffraction experiments was grown by slow evaporation from petroleum ether–acetone (9:1, v/v). The crystal data and structure refinement detail of the compound were given in (Table 1). Specified hydrogen bonds were shown in (Table 2) and (Figure 2). The molecular structures with the atom-numbering scheme and unit cells packing figures were shown in (Figures 3 to 4). The characteristic IR absorption bands observed (Figure 5) were consistent with the functional groups present in the compound. The UV scanned spectrum showed a maximum absorption took place at wavelength of 220 and 275 nm (Figure 6). Based on the above analysis, the compound was identified as ethyl 4-hydroxy-3,5-dimethoxy-benzoate which was firstly isolated from the genus *Scutellaria*.

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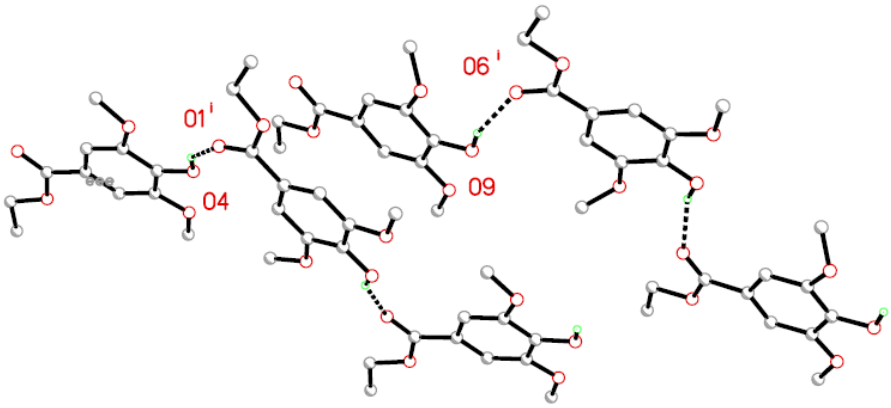

Figure 2. The hydrogen-bonded of the compound.

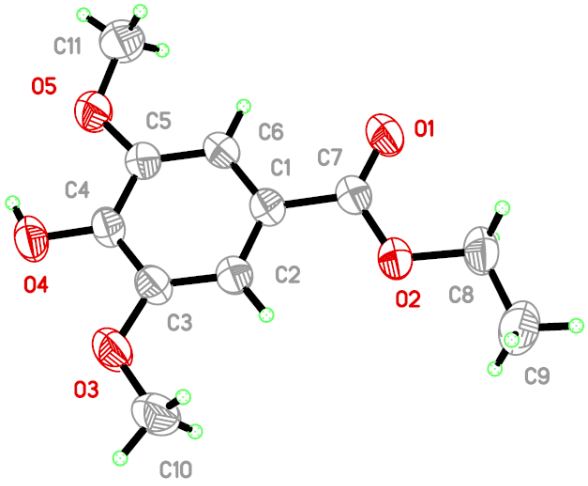

Figure 3. Ethyl 4-hydroxy-3,5-dimethoxy-benzoate. Molecular formula.$C_{11}H_{14}O_5$. Physical description: white granular crystal (petroleum ether–acetone, 9:1, v/v). Melting point: 88-90°C.

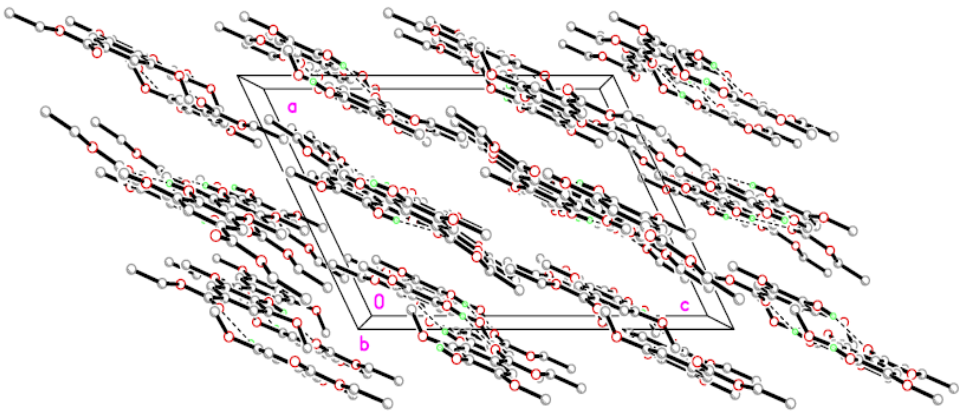

Figure 4. The crystal structure of the compound, showing the packing mode of the dimmers.

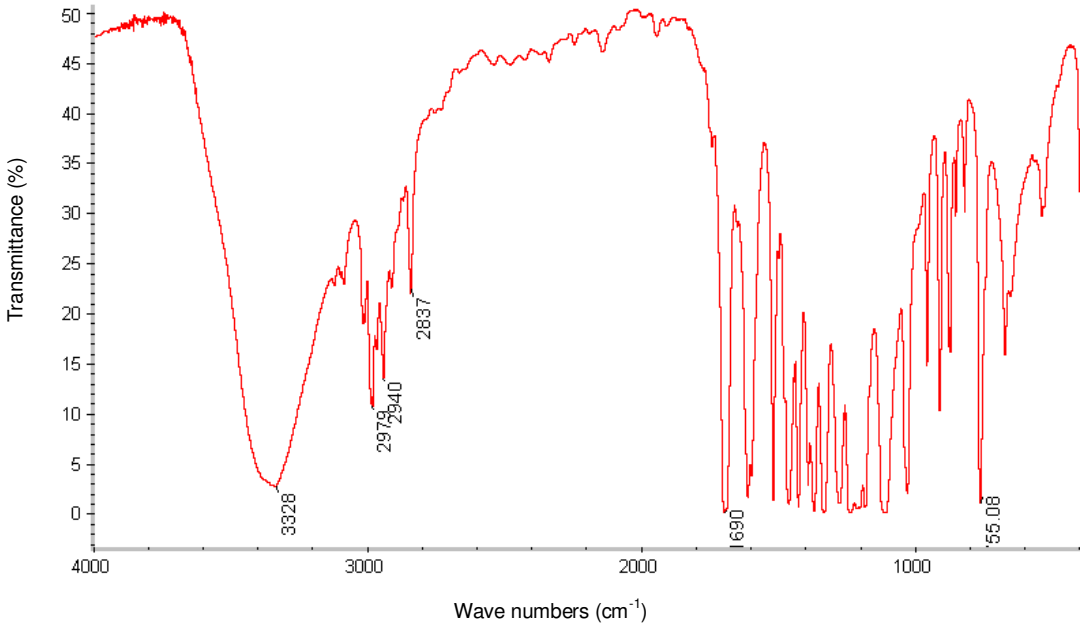

Figure 5. The IR spectrum of the compound.

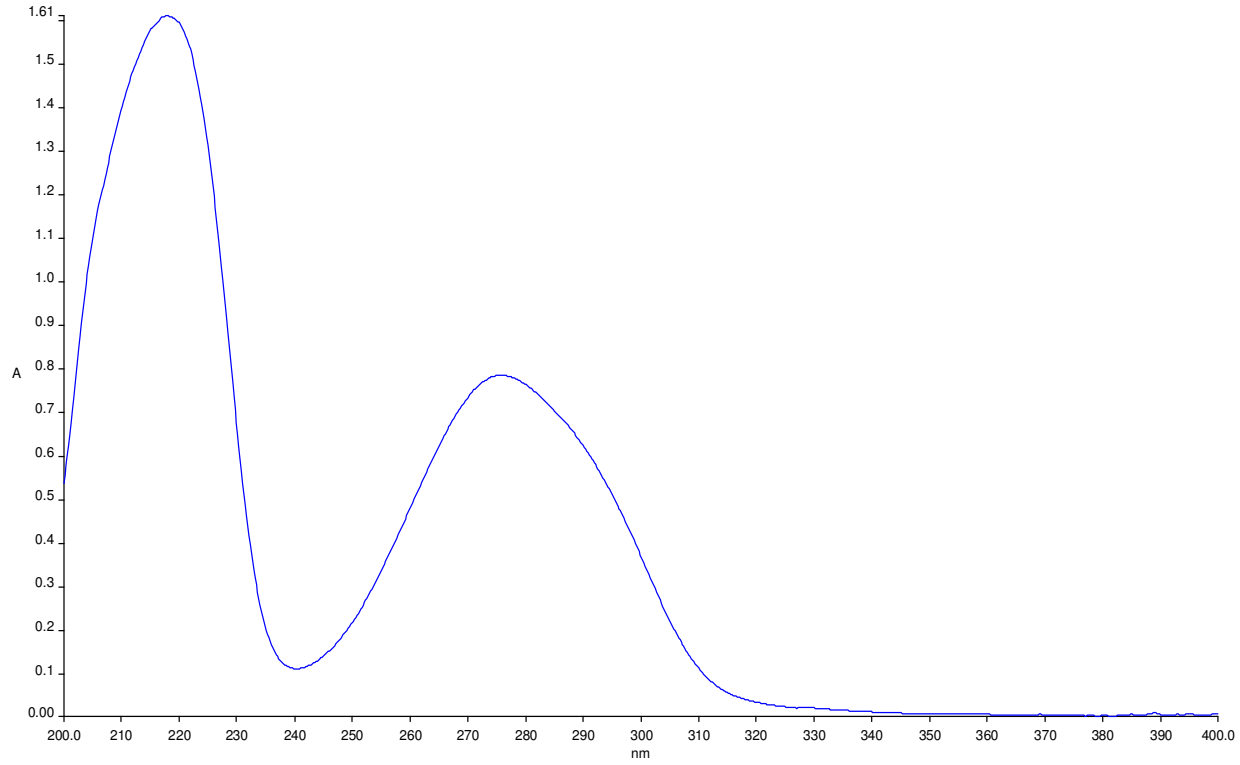

Figure 6. UV spectrum of the compound.

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