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Bothriodumin, a shikonin derivative from *Bothriospermum secundum* Maxim.

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A novel shikonin derivative, named bothriodumin was isolated from the alcoholic extract of the whole plant of *Bothriospermum secundum* Maxim. And its structure was characterized on the basis of spectroscopic methods such as 1D-, 2D-NMR and HR-ESIMS techniques.

1. Introduction

Species of Boraginaceae family are known to contain naphthoquinones, phenolic acids, alkaloids and other constituents (Huang et al. 2000). Among them, naphthoquinones are the most attractive constituents as having many pharmacological effects, such as antitumor, antibacterial, antifungal, anticoagulant and antioxidant properties (Hu et al. 2006; Huang et al. 2000; Papageorgiou et al. 1999). For these reasons, the investigation on Boraginaceae family has been one of the subjects of our laboratory (Jin et al. 2004, 2007). The genus *Bothriospermum* (Boraginaceae) comprises five species distributed throughout the tropic and temperate zone of Asia (Ma et al. 2003, 2005; Wu et al. 1999). *B. secundum* has been used as important folk medicine for dispelling wind, detoxification, and as an insecticidal (Jiangsu New Medical College 1975). In order to find active constituents, the phytochemical investigation on *B. secundum* was carried out for the first time, and one novel compound was obtained from the alcoholic extract. We herein report the isolation and structural elucidation of this compound.

2. Investigations, results and discussion

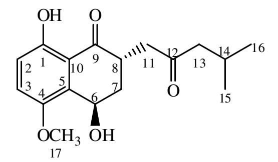
From the alcoholic extract of the whole plant of *Bothriospermum secundum* Maxim., a novel shikonin derivative, named bothriodumin (**1**) was isolated by repeat column chromatography.

Compound **1** was obtained as a brown gum, $[\alpha]_{20}^{D}$ −3° (C 1.0, $CHCl_3$). The molecular formula was assigned as $C_{17}H_{22}O_5$ on the basis of the $[M + Na]^+$ at m/z 329.1346

Table: ^{1}H-(400.13 MHz) and ^{13}C NMR (100.62 MHz) data, ^{1}H-^{1}H COSY and HMBC for compound 1

No.	δ_H	δ_C	COSY	HMBC
1	–	155.89 s	–	H-2, H-3
2	6.89, d (9.2)	118.05 d	H-3	H-3
3	7.11, d (9.2)	28.90 d	H-2	H-2
4	–	148.88 s		H-2, H-3, CH_3O-17
5	–	130.48 s	–	H-3, H-6
6	5.24, t (3.2)	61.10 d	H -7	H_2-7
7	2.22 (m)	34.80 t	H-6, H-8	H-8, H-11α, H-11β
8	3.74 (m)	37.44 d	H_2-7, H-11α, H-11β	H_2-7, H-11α, H-11β
9	–	205.46 s	–	H_2-7, H-11α H-11β, H-8
10	–	115.14 s	–	H-2, OH-1
11α	2.89, dd (6.0, 15.6)	42.65 t	H-8, H-11β	H-7, H-8
	2.64, dd (6.0, 15.6)		H-8, H-11α	
12	–	208.20 s	–	H-11α, H-11β, H_2-13
13	2.37, dd (2.0, 6.8)	52.03 t	H-14	H-14, H_3-15, H_3-16
14	2.15 (m)	24.67 d	H-13, H_3-15, H_3-16	H-13, H_3-15, H_3-16
15	0.93, dd (0.8, 6.8)	22.60 q	H-14, H_3-16	H-13, H-14, H_3-16
16	0.93, dd (0.8, 6.8)	22.54 q	H-14, H_3-15	H-13, H-14, H_3-15
17-OCH_3	3.84, s	56.44 q	–	–
1-OH	11.67, s	–	–	–

Assignments were aided by spin splitting patterns, DEPT, HMQC, HMBC experiments, and chemical shift values (δ. The δ values are in ppm and are referenced to either the residual $CHCl_3$ (7.26 ppm) or $CDCl_3$ (77.0 ppm) signals



1

Fig. 1: The molecular structures of compound **1**

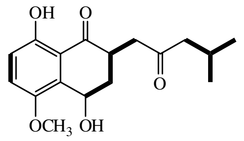


Fig. 2: Structural parts of COSY in **1** (Bold bonds)

(calc. 329.1359 for $C_{17}H_{22}O_5Na$) in its HR-ESIMS, which was supported by the results, 3 × CH_3, 3 × CH_2, 5 × CH and 6 × C from ^{13}C NMR analysis combined DEPT experiment. The IR spectrum showed absorption bands for hydroxyl (3419 cm^{-1}), carbonyl (1710 cm^{-1}), phenyl groups and double bond (1643, 1587 cm^{-1}). The ^1H and ^{13}C NMR spectrum (Table) indicated the presence of an aromatic hydroxyl proton (δ_H 11.67, s, 1 H), an aromatic methoxyl group ((δ_H 3.89, s, 3 H) and δ_C (56.44, q)), an allylic oxygenated methine (δ_H (5.24, t, 1 H, $J = 3.2$ Hz) and δ_C (61.10, d)), and two unsaturated carbonyls (δ_C 208.20 and 205.46). Also a typical pattern for 1,2,3,4-tetra substituted aromatic ring was observed by six aromatic carbons and two doublets of an AB spin system (δ_H 7.11 and 6.89, d, $J = 9.2$ Hz). By comparing the spectral data of **1** with those of reported data (Buchanan et al. 1996; Jin et al. 2004), **1** appears to be a naphthalene derivative. The structure of **1** could be further confirmed by 2D NMR spectroscopic analysis.

In the ^1H-^1H COSY spectrum of **1**, there were some significant cross-peaks (Table), suggesting the presence of three main partial structures (see Fig. 2). The HMBC correlations (see Fig. 3) of H-11 (δ_H 2.89 and 2.64) and H-13 (δ_H 2.37) with C-12 (δ_C 208.20), H-8 (δ_H 3.74) with C-9 (δ_C 205.46), as well as H-7 (δ_H 2.22) with C-5 (δ_C 130.48) and C-6 (δ_C 61.60) indicated that C-13 and C-11 were attached to C-12 and C-8 was connected with C-9, and C-5 was connected with C-6. Furthermore, the correlations of the hydroxyl proton (δ_H 11.67) with C-1 (δ_C 155.89) and methoxyl proton H-17 (δ_H 3.84) with C-4 (δ_C 148.88) revealed that the hydroxyl group was attached to C-1 and the methoxyl group was attached to C-4 respectively. In the NOESY spectrum of **1**, there was no correlation for H-6 with H-8, suggesting C-6 and C-8 with different relative configuration. By the minimize energy calculations of the most stable optical isomer of **1** under Chemdraw 10.0, we confirmed that 6-OH and 8-H were both for β-oriented. Thus, the structure of compound **1** was established, it was a derivative of shikonin and named as bothriodumin after its resource (*Bothriospermum secundumine*).

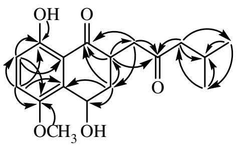
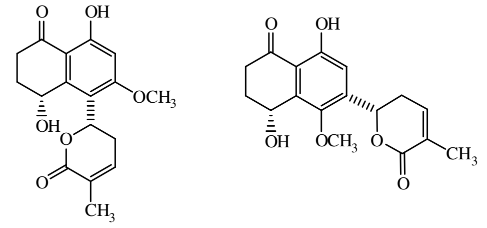


Fig. 3: HMBC correlations of **1** (From H to C)



a **b**

Fig. 4: Structure of lappulanocarpine A reported (**a**) and corrected (**b**)

As soon as structure of **1** was confirmed, we found that the compound lappulanocarpine A (see Fig. 4a) isolated in a previous study (Jin et al. 2004) must also be a shikonin derivative and the structure of lappulanocarpine A should be corrected as shown in Fig. 4b.

3. Experimental

3.1. Equipment

Melting points were determined with an X-4 Digital Display Micro-Melting point apparatus, and are uncorrected. Optical rotations recorded in $CHCl_3$ using a Perkin Elmer 241 polarimeter. UV-spectra were measured on a Spect 50-UV/Vis spectrophotometer in MeOH solution (Analytic Jena AG). IR spectra were measured on an FTS165-IR instrument (Bio-Rad, USA). ^1H NMR (400.13Hz), ^{13}C NMR (100.62Hz) and 2D-NMR were recorded on a Varian INOVA-400 FT-NMR spectrometer (USA) in $CDCl_3$ with TMS as internal standard. HR-ESIMS was recorded on a Bruker APEX II. Silica gel (200–300 mesh) used for column chromatography and silica gel (GF_{254}) for TLC are supplied by the Qingdao Marine Chemical Factory in China. Spots were detected on TLC by visualization under UV light or by spraying with 98% H_2SO_4-EtOH (v/v, 5 : 95) followed by heating at 110 °C.

3.2. Plant material

The plant material (No. 2001–09) was collected from Huining county, Gansu province of P.R. China and was identified by adjunct Prof. Ji Ma, Faculty of Pharmacy, First Military Medical University of PLA, Gangzhou, P.R. of China. A specimen has been deposited at our Lab.

3.3. Extraction and isolation

Air-dried and grounded whole plant of *B. secundumine* Maxim (2.0 kg) was extracted seven times with 95% EtOH at room temperature (7 × 24 h), and the solvent was removed under reduced pressure to give a residue (120 g). The residue was subjected to chromatography on a silica gel column using petroleum ether (60–90 °C) with increasing volumes of acetone as eluent to give four fractions (Fr. 1–Fr. 4). In which, Fr. 3 (v/v, from 10 : 1 to 5 : 1) was subjected to silica gel chromatography and eluted with chloroform-ethyl acetate (v/v, 20 : 1) to give sub-fractions (Fr. A–D). And Fr. D was subjected to silica gel column using petroleum-acetone (v/v, 8 : 1) as eluent to produce compound **1** (7 mg).

3.4. Bothriodumin (1)

Brown gum. $[\alpha]_{20}^{D}$, −3° (C 1.0, $CHCl_3$). IR (KBr, cm^{-1}): 3419, 2956, 2930, 2872, 1710, 1643, 1614, 1587, 1473, 1442, 1367, 1289, 1265, 1198, 1184, 1057, 1039, 912, 824, 791, 729. HR-ESIMS: revealed m/z = 329.1346, requires m/z = 329.1359 for $C_{17}H_{22}O_5Na$. ^1H and ^{13}C NMR: see Table.

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