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Biocatalyzed Cross-Coupling of Sinomenine and Guaiacol by *Antrodiella semisupina*

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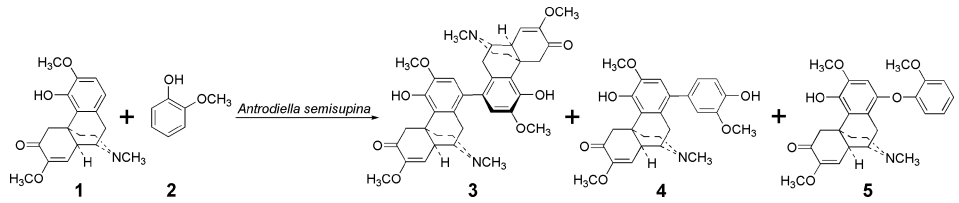
ABSTRACT



In search of more potent derivatives of sinomenine (1), a clinically available natural alkaloid for the treatment of rheumatoid arthritis (RA), biocatalyzed cross-coupling of sinomenine and guaiacol (2) by *Antrodiella semisupina*, provided two unique C–C coupled (3 and 4) and one C–O linked (5) novel metabolites. The structures of the metabolites were elucidated by means of MS, 2D NMR techniques and X-ray analysis. 4 exhibited more potent inhibitory activity on IL-6 production than 1 in human synovial sarcoma cell (SW982), and 5 stimulated IL-6 production.

Natural products continue to provide pharmaceutics with a plethora of valuable candidates for drug discovery. Microorganisms and their enzymes have been proven to possess versatile biocatalysts and are widely believed to be a powerful tool not only for generation of new, active, and less toxic derivatives from bioactive natural products but also for effective synthesis of intermediates of new or improved drugs.[1,2] Sinomenine (7,8-didehydro-4-hydroxy-3,7-dimethoxy-17-methylmorphinan-6-one, **1**), a natural morphine-type alkaloid, was isolated from the Chinese medicinal plant *Sinomenium acutum* (Thunb.) Rehd. et Wils. with a high content.[3,4] It has been used for the treatment of rheumatic disease including rheumatoid arthritis (RA) for a long time in clinic,[5] and a variety of bioactivities, such as anti-inflammation, immuno-suppression, antiarthritis, and cardioprotection have also been reported.[6−9] Furthermore, a number of sinomenine derivatives have been chemically synthesized for anti-inflammatory purposes.[10]

Our previous work has been focused on structural modification by a chemical method.[11] As a part of a research project to explore new and more bioactive derivatives from sinomenine using microbial transformation for drug discovery, we screened numbers of fungi and discovered 3 strains

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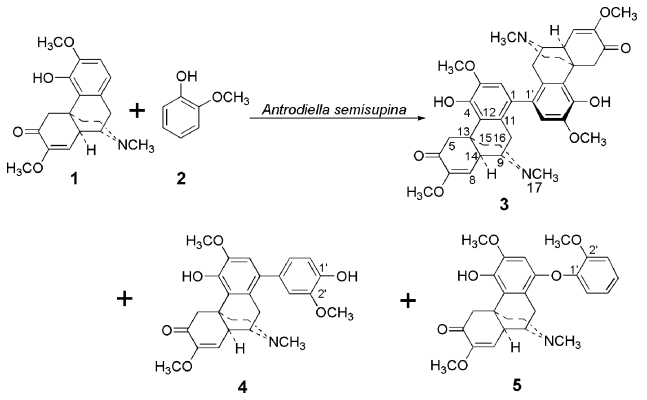
Scheme 1. Cross-Coupling of Sinomenine **1** and Guaiacol **2** Biotransformed by *Antrodiella semisupina*



that are capable of converting sinomenine to metabolites, one of them, *Antrodiella semisupina* (Berk. and Curt.) Ryv., showed the highest transformation capacity.

There are several reports on peroxidase-catalyzed aryl coupling.[12] To the best of our knowledge, no work has been performed on two or more substrates. In this paper, aimed at the discovery of novel structural products that are difficult to produce chemically, sinomenine (**1**) and guaiacol (2-methoxyphenol, **2**) were subjected synchronously to *A. semisupina*, and three novel compounds including two C−C coupled metabolites were obtained. This is the first report that biocatalyzed coupling on multi-substrates has been conducted.

When **1** was incubated with *A. semisupina* under liquid conditions for 96 h, a single product **3** with about 20% yield based on the starting material (**1**) was obtained. This result revealed that *A. semisupina* possessed a high capacity of microbial transformation and provided an encouraging clue to further introduce a small molecule into the sinomenine skeleton. In our previous research, introduction of a bioactive molecule with anti-inflammatory effect at N-atom of the sinomenine skeleton provided a great enhancement of the activity.[11] Guaiacol (**2**) has been widely used for medicinal practice as an anti-inflammatory and antibacterial agent;[13,14] therefore, it was incorporated into the research. As expected, coincubation of **1** and **2** with *A. semisupina* provided two C−C coupled metabolites (**3**, 20% yield and **4**, 13% yield) and a C−O linked one (**5**, 5% yield) (Scheme 1).

Compound **3** was obtained as a colorless hexahedral crystal ($CHCl_3/CH_3OH/EtOAc$ = 80:15:5), mp. 220−222 °C, and showed $[\alpha]^{25}_D$ = +12.9° (c = 0.45, CH_3OH). EI-MS and

Table 1. ^1H NMR and ^{13}C NMR Spectral Data (δ in ppm, J in Hz) of Compounds **3**−**5**[a]

	3		**4**		**5**	
no[b]	H	C	H	C	H	C
1		130.7		131.8		144.1
2	6.27 (s)	110.7	6.56 (s)	111.0	6.44 (s)	103.1
3		144.8		144.5		147.5
3-OCH_3	3.75 (s)	56.0	3.80 (s)	56.0	3.73 (s)	56.1
4		143.8		143.8		141.7
5	2.49 (d, 15.6)	49.3	2.49 (d, 15.6)	49.1	2.47 (d, 15.7)	49.0
	4.41 (d, 15.6)		4.42 (d, 15.6)		4.37 (d, 15.7)	
6		193.8		194.2		193.7
7		152.5		152.3		152.4
7-OCH_3	3.51 (s)	54.8	3.52 (s)	54.9	3.49 (s)	54.8
8	5.43 (d, 2.4)	115.0	5.46 (d, 1.9)	115.2	5.39 (d, 1.8)	114.8
9	3.08 (brt, 4.0)	56.4	3.12 (brt, 4.0)	56.6	3.19 (brt, 5.8)	55.8
10	2.42 (dd, 18.6, 4.0)	23.9	2.75 (brd, 18.6)	23.4	3.00 (d, 18.5)	18.5
	2.32 (brd, 18.6)		2.41 (dd, 18.6, 4.0)		2.36 (dd, 18.5, 5.8)	
11		127.8		127.7		122.5
12		123.3		122.5		123.4
13		40.9		40.8		40.4
14	2.99 (brs)	45.8	3.01 (dd, 4.0, 1.9)	45.6	3.03 (brd, 5.8)	45.6
15	2.02 (dt, 12.2, 3.4)	35.9	2.02 (dt, 12.4, 3.2)	35.8	2.00 (dt, 12.4, 3.4)	35.6
	1.91 (td, 12.2, 4.3,)		1.89 (td, 12.4, 4.4)		1.93 (td, 12.4, 4.4)	
16	2.16 (td, 12.0, 3.4)	47.2	2.10 (td, 12.2, 3.2)	47.4	2.14 (td, 12.0, 3.4)	47.0
	2.57 (ddd, 12.0, 4.3, 2.1)		2.57 (ddd, 12.2, 4.4, 1.5)		2.58 (ddd, 12.0, 4.4, 1.8)	
17	2.35 (s)	43.0	2.31 (s)	43.8	2.36 (s)	42.7
1′				144.6		147.5
2′				146.3		149.1
2′-OCH_3			3.89 (s)	55.9	3.95 (s)	56.0
3′			6.68 (s)	111.7	7.00−6.96 (overlapped)	112.2
4′				134.3	7.00−6.96 (overlapped)	122.3
5′			6.67 (dd, 8.4, 1.8)	121.9	6.75 (m)	120.7
6′			6.94 (d, 8.4)	114.3	6.36 (dd, 8.5, 1.1)	114.6

[a] Recorded in Chloroform-d. [b] δ of 1′−17′ of compound **3** are the same as δ of 1−17, respectively.

high resolution (HR) EI-MS exhibited an ion peak at *m*/*z* 656 $[M]^+$ and 656.3099 (calcd for 656.3098), indicating the molecular formula was $C_{38}H_{44}N_2O_8$. Extensive analyses of ^1H and ^{13}C NMR spectra (Table 1) together with the use of $^1H-^1H$ COSY and HMQC data of **3** revealed that the signal patterns of **3** were quite similar to those of **1** except that only one aromatic proton signal was observed instead of the characteristic AB pair of **1**. However, the even molecular weight of 656 implied the presence of an even number of nitrogen atoms. These results suggested **3** might be a symmetrical dimer of **1**, in which the connection position should be at the aromatic ring. Further HMBC experiment confirmed the substitution point was at C-1. Thus, the planar structure of **3** was deduced; hereto, the stereochemistry of **3** was still unclear. Unfortunately, the NOESY and CD data could not provide any available clues. In addition, several papers have reported the disinomenine, but no reliable information on its exact structure, especially stereo-structure, was provided.[15] Therefore, much effort was made to successfully produce fine crystals to determine the structure directly by an X-ray crystallographic analysis. Fortunately, the result clearly confirmed the structure of **3** including stereo-structure (Figure 1), a dimer of **1** as shown in Scheme 1.

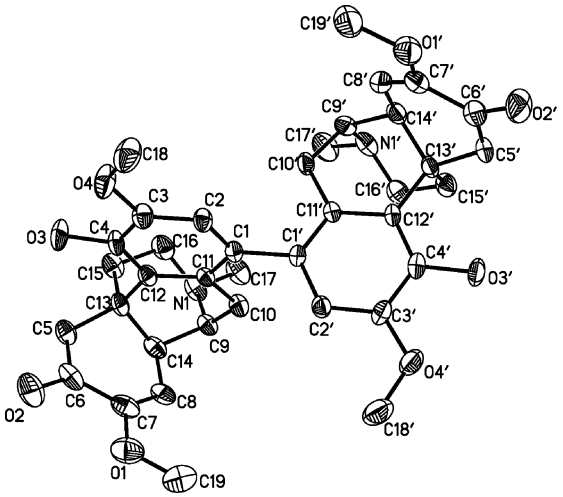


Figure 1. X-ray crystal structure of the compound **3** showing the crystallographic numbering.

Compound **4** was obtained as a white powder, showed mp. 151−153 °C, $[\alpha]^{25}_D = +14.5°$ ($c = 0.4$, CH_3OH), and its molecular formula was determined to be $C_{26}H_{29}NO_6$ [$(M)^+$ 451.1971, calcd for 451.1995] in HR-ESI-MS, which meant the existence of an odd number of nitrogen atoms. The ^1H NMR spectrum clearly showed signals including a single aromatic proton as **3** from sinomenine skeleton (Table 1). Moreover, a set of 1,3,4-trisubstituted phenyl group and a methoxyl group assignable to guaiacol were observed. Detailed analyses of $^1H-^1H$ COSY and HMQC spectra suggested that **4** should be composed of sinomenine and guaiacol moieties. To determine the connectivity of the two moieties, HMBC was measured. Clear long-range correlation between C-4′ and H-2, C-1 and H-5′ proposed that C-4′ of guaiacol attached to C-1 of sinomenine via a C−C bond. From forenamed findings, the structure of **4** was deduced as shown in Scheme 1.

Compound **5** was isolated as a slightly yellow powder with a molecular ion peak at *m*/*z* 451.1962 $[M]^+$ (calcd for $C_{26}H_{29}NO_6$, 451.1995) in HR-ESI-MS spectrum and $[\alpha]^{25}_D = +1.8°$ ($c = 0.45$, CH_3OH). Detailed analysis of ^1H NMR spectrum with $^1H-^1H$ COSY and HMQC revealed that **5** possessed the same sinomenine moiety as **4**; however, aromatic protons assignable to guaiacol moiety displayed 1,2-disubstutited pattern which is the same as those of guaiacol. Moreover, in ^{13}C NMR spectrum of **5** together with analysis of HMBC spectrum, C-1 (δ 144.1) displayed a marked downfield shift compared with that (δ 131.8) of **4**. This evidence led us to suppose that sinomenine and guaiacol moieties were connected via a ether bond, which was further supported by the result of acetylation of **5**, a monoacetate of OH at C-4 was obtained. On the basis of these data, compound **5** was elucidated as shown.

The possible mechanism in the formation of C−C bonds to give unique structures is of great interest. From the structures of compounds **3**−**5**, most probably, oxidation of **1** or **2** generated oxygen radicals at OH group, and then the oxygen radicals were delocalized to form carbon-located radicals. Subsequent coupling of these radicals produced C−C or C−O metabolites. It is reasonable that some enzymes presented in *A. semisupina* might catalyze this type of reaction.[12] To verify the role of the OH group in the formation of the metabolites, 4-acetyl sinomenine was subjected to biotransformation followed the same protocol. The result showed that no **3**, **4**, and **5** were detected by high-performance thin layer chromatography (HPTLC) analysis. This experiment strongly suggested that the 4-OH is a crucial group during the formation the metabolites. However, as mentioned previously,[15] sinomenine could be oxidized by silver nitrate to form two 1,1′-disinomenines (ratio = 1:1) although the structures were not determined. Our experiments revealed that the MnO_2 catalyzed reaction of **1** and **2**

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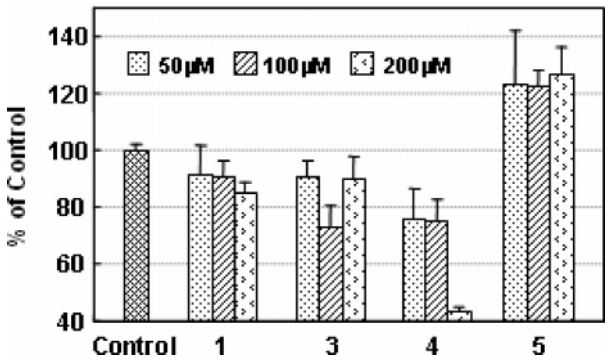

Figure 2. Inhibitory effects of sinomenine (**1**) and the metabolites (**3**, **4**, and **5**) on IL-6 production in SW982 cells. Control: no added compounds; **1**, **3**−**5**: added each compound, respectively. SW982 cells were cultured for 2 d with 1 ng/mL of IL-1β in the presence or absence of compounds. IL-6 concentrations in culture supernatants were measured by ELISA. Each value represents the mean ± S.D. from triplicate cultures. All compounds showed no significant inhibitory effects on cell growth or morphology at 200 μM.[18]

provided main compound **5** and minor two 1,1′-disinomenines; no **4** was detected. Furthermore, horseradish peroxidase (HRP) catalyzed reaction of **1** or **1** and **2** provided the same results, only two 1,1′-disinomenines were obtained. These results implied that there should be an enzyme system presented in *A. semisupina* responsible for the unique C−C couplings with stereoselectivity and enzymatic exclusivity, which was different from the conversions via HRP and chemical reaction. Further research is essential to clarify it.

Human synovial sarcoma cells (SW982) are characterized by high levels of expression of proinflammatory cytokines, cyclooxygenase (COX)-2, rostaglandin (PG)E2, interleukin (IL)-6, and matrix metalloproteinases (MMPs) genes in the presence of IL-1β stimulation.[16] The cytokines are thought to participate in the pathogenesis of numerous inflammatory diseases, including rheumatoid arthritis (RA).[17] Therefore, the metabolites together with **1** were tested for their inhibitory activity on IL-1-induced IL-6 production in SW982 cells. As shown in Figure 2, **4** displayed much more potent activity than **1**; however, **5** stimulated the IL-6 production.

This is the first demonstration that multi-substrate couplings were generated by fungi, and sinomenine, a clinically available drug, was modified to generate unique C−C coupled derivatives. Because microbial transformations of natural products is a powerful tool to produce novel drugs and key intermediates that are difficult to be synthesized chemically, our results provide an efficient means by which the substrate can be selectively modified by a second one. Further expansion of the unique C−C cross-coupling reactions, enzymological research, and bioassays are currently in progress.

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Supporting Information Available: Experimental details for the incubation, separation of metabolites from incubation mixture, spectroscopic data for metabolites, and bioactivity for related compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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