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pH-Dependent, Stereoselective Dimerization of Sinomenine

Zhang-Shuang Deng, Yu Zhao, Cui-Cui He, Jie Jin, Yun-Mian He, and Jian-Xin Li*

Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing, 210093, P. R. China

lijxnju@nju.edu.cn

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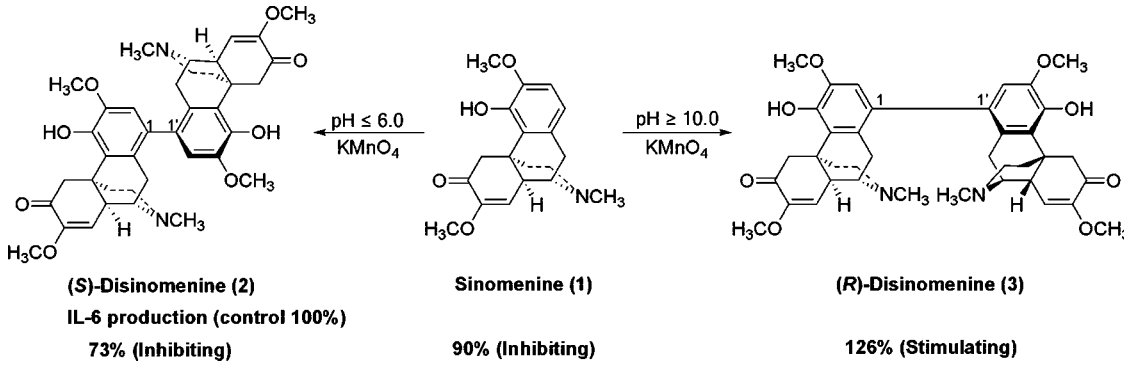
ABSTRACT



In a continuing study on discovery of more potent derivatives of sinomenine (1), a clinically available alkaloid for rheumatoid arthritis (RA) treatment, oxidation of sinomenine provided two unique stereoisomers, disinomenines 2 and 3. The structure of 3 was determined by MS, NMR, and X-ray analysis. The formation of 2 and 3 via oxidation of sinomenine by potassium permanganate ($KMnO_4$) exhibited a pH-dependent stereoselectivity. The bioassay results using human synovial sarcoma cells (SW982) showed that 2 inhibited, while 3 stimulated, IL-6 production.

Rheumatoid arthritis (RA) is a chronic and systemic rheumatic disease that is characterized by the progressive destruction of articular cartilage and bone.[1–6] Sinomenine [(9α,13α,14α)-7,8-didehydro-4-hydroxy-3,7-dimethoxy-17-methylmophinan-6-one], a natural morphine-type alkaloid isolated from the stem of the Chinese medicinal plants *Sinomenium acutum* and *Sinomenium acutum* var. *cinereum*, is clinically available for the treatment of rheumatic disease, especially RA, and a variety of bioactivities such as anti-inflammation, abirritation, immunosuppression, and arthritis amelioration have also been reported.[7–13] In order to explore novel structural and more bioactive sinomenine derivatives for the discovery of anti-RA agents, we recently reported a stereoselectively and exclusively biocatalyzed C−C cross-coupling reaction of sinomenine and guaiacol by a fungus, *Antrodiella semisupina*.

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Table 1. ^{1}H NMR and ^{13}C NMR Spectral Data (δ in ppm, J in Hz) of Compounds **2**[a] and **3**[a]

	(*S*)-disinomenine (**2**)[c]		(*R*)-disinomenine (**3**)	
no.[b]	H	C	H	C
1		130.7		130.8
2	6.27 (s)	110.7	6.45 (s)	109.5
3		144.8		145.2
3-OCH_3	3.75 (s)	56.0	3.81 (s)	56.1
4		143.8		144.0
5	2.49 (d, 15.6)	49.3	2.51 (d, 15.7)	48.9
	4.41 (d, 15.6)		4.43 (d, 15.7)	
6		193.8		193.6
7		152.5		152.5
7-OCH_3	3.51 (s)	54.8	3.51 (s)	54.5
8	5.43 (d, 2.4)	115.0	5.27 (d, 1.3)	114.0
9	3.08 (brt, 4.0)	56.4	3.03−3.15 (overlapped)	56.5
10	2.42 (dd, 18.6, 4.0)	23.9	2.53 (brd, 18.7)	23.6
	2.32 (brd, 18.6)		1.83 (dd, 18.7, 5.1)	
11		127.8		127.5
12		123.3		122.9
13		40.9		40.4
14	2.99 (brs)	45.8	3.03−3.15 (overlappled)	45.0
15	2.02 (dt, 12.2, 3.4)	35.9	2.03 (m)	35.3
	1.91 (td, 12.2, 4.3)		1.97 (td, 12.7, 4.0)	
16	2.16 (td, 12.0, 3.4)	47.2	2.13 (td, 11.2, 2.6)	47.4
	2.57 (ddd, 12.0, 4.3, 2.1)		2.66 (brd, 11.2)	
17	2.35 (s)	43.0	2.36 (s)	42.8

[a] Recorded in chloroform-*d*. [b] δ of 1′ −17′ of compounds **2** and **3** are the same as δ of 1−17, respectively. [c] The data of **2** are taken from ref 14.

One of the metabolites, (*S*)-disinomenine (**2**), showed a potent inhibitory activity on IL-6 production.[14] Further experiments on the oxidation of sinomenine catalyzed by MnO_2 and horseradish peroxidase (HPR) provided **2** and another product **3** (**2**:**3** = 1:1 ratio), while it left us a puzzle to determine the structure of **3**.

In continuing studies aimed at clarifying the structure of **3** and selective synthesis of the disinomenines, oxidative dimerization of sinomenine catalyzed by other chemical oxidants such as $AuCl_3$, $AgNO_3$, $K_3Fe(CN)_6$, and $FeCl_3/H_2O_2$, followed by silica gel column chromatography ($CHCl_3$−MeOH = 9:1), provided the same results as those of MnO_2 and HPR. Manganese reagents such as $Mn(OAc)_3$ and $KMnO_4$ were also found to be effective oxidants. Interestingly, compounds **2** and **3** are two very unique stereoisomers of disinomenine as shown in Figure 1. In this paper, we report the stereostructure of **3** and a pH-dependent, stereoselective dimerization of sinomenine.

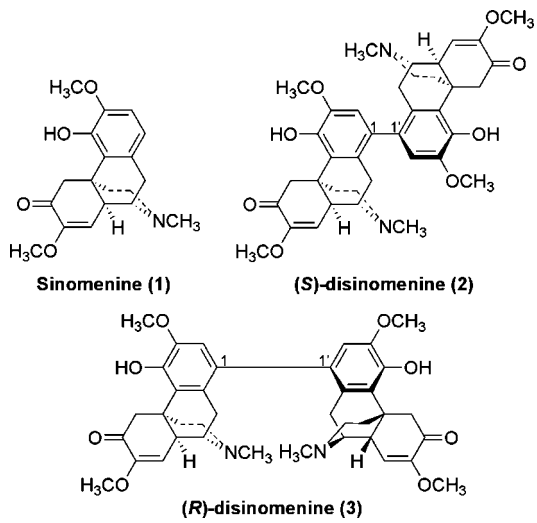

Figure 1. Chemical structures of sinomenine (**1**), (*S*)-disinomenine (**2**), and (*R*)-disinomenine (**3**).

Compound **3** was obtained as a colorless hexagonal crystal (CH_2Cl_2), mp 231−233 °C, and showed $[\alpha]^{25}_{D}$ = +10.5 (c = 0.4, CH_3OH). EI-MS and high-resolution (HR) EI-MS exhibited ion peaks at m/z 656 $[M]^+$ and 656.3071 (calcd for 656.3098), indicating the molecular formula was $C_{38}H_{44}N_2O_8$. The ^{1}H and ^{13}C NMR spectra (Table 1) of **3** analyzed with the aid of ^{1}H−^{1}H COSY and HMQC were closely similar to those of **2** except that the proton signals at C-9 and C-2 in **3** displayed downfield shifts and the proton signal at C-9 overlapped with that of C-14. Furthermore, the only one aromatic proton signal in **3** together with the molecular formula suggested that **3** should be a symmetrical dimer of **1**, the same as **2**. HMBC correlation between H-2 and C-4 confirmed that the connection position of two sinomenine moieties was at C-1. Based on the above findings, it was concluded that **3** should be a stereoisomer of **2** with the exact same planar structure, yet determining its stereo-

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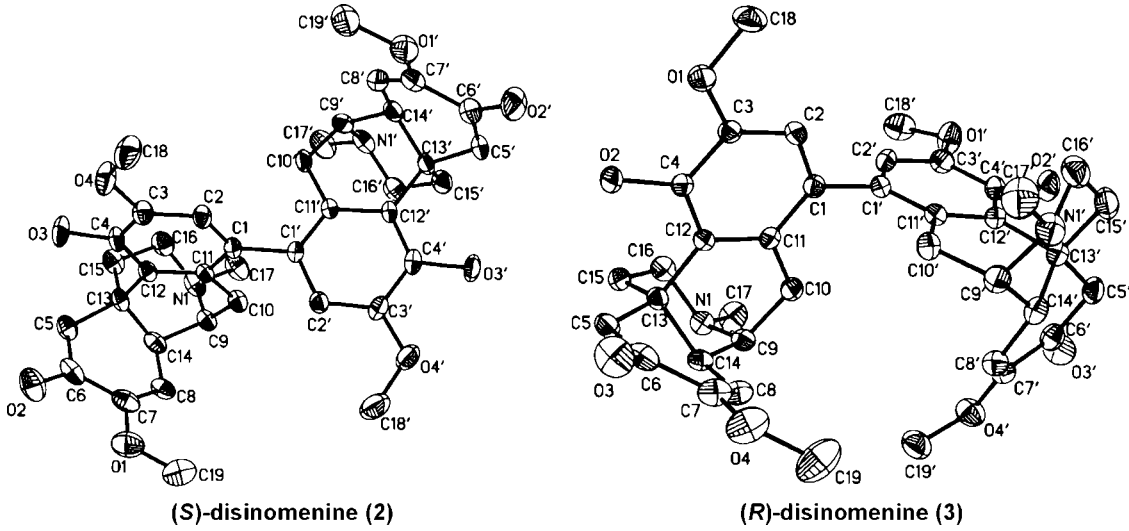

Figure 2. X-ray crystal structures of **2** and **3** showing the crystallographic numbering and the data of **2** from ref 14.

structure was a big challenge. After a great effort to successfully make fine crystals, the stereostructure of **3** was established by an X-ray crystallographic analysis. Interestingly, the result revealed that **3** was an unique stereoisomer of **2** with a different angle around the 1,1′-C−C axis (Figure 2). Circular dichroism (CD) of **2** and **3** are nearly similar except that compound **2** exhibited a positive Cotton effect at 296.7 nm, indicating the difference on regiochemistry of biphenyl linkage.

To the best of our knowledge, several papers have already reported disinomenines during the past 80 years, but no exact structures, especially on their stereostructures, were provided. Goto et al. reported the studies on bimolecular sinomenine, disinomenine, and Ψ-disinomenine and postulated that two dimers were stereoisomers with restricted rotation around their 1,1′-diphenyl axis because of steric hindrance.[15] Brossi et al. speculated that a factor hindering free rotation might be derived from their strong affinity to solvents and these crystalline dimers contain tightly bound solvents of crystallization, which was the reason they failed X-ray analysis.[16] Recently, Qin isolated **2** from *S. acutum* and misdetermined its stereostructure based on Brossi's hypothesis.[17] Fortunately, we first elucidated the stereostructures of **2** and **3** and completely assigned all of the ^{1}H and ^{13}C NMR signals.

Although the causes of RA are not completely understood, proinflammatory cytokine interleukin-6 (IL-6) proved to be involved in the pathogenesis of the disease.[18] Constitutive overproduction of IL-6 is thought to play important pathological roles in RA, and elevated IL-6 levels show close correlation with disease activity, radiographic joint damage, and RA-associated anemia.[19] Thus, the effects of sinomenine, **2**, and **3** on IL-6 production in IL-1β-stimulated human synovial sarcoma cells (SW982) were evaluated.[20] Compounds **2** and **3** showed a very interesting structure−activity relationship: **2** exhibited more potent inhibitory activity on IL-6 production than sinomenine at 100 μM.[14] Conversely, **3** stimulated IL-6 production (Figure 3).

As previously described, the chemical dimerization of sinomenine provided a mixture of **2** and **3** in an approximately 1:1 ratio. Due to the stronger bioactivity of **2** and further expansion of dimers of sinomenine derivatives, an attempt was made to stereoselectively synthesize **2** and **3**. As shown in Figure 4, the oxidation of sinomenine by $KMnO_4$ in an aqueous solution exhibited a stereoselectivity at different pH values. When the pH was under 5, the main product was (*S*)-disinomenine (**2**) (93.8%) with only about 6.2% of (*R*)-disinomenine (**3**). When the pH was above 10, the oxidative reaction yielded **3** at 97.7% together with **2** at only 2.3%. However, at lower pH values, the total yield rates of two dimers were decreasing, which could be attribute to the strong oxidative effect of potassium permanganate under acidic conditions. Both **2** and **3** without oxidants under acidic or basic conditions were stable at room temperature, and no

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interconverted products were obtained. Further experiments to get better yield of **2** discovered that the stereoselectivity of **2** could be increased under a microfluidic condition, the details is still undergoing.

In summary, we have clarified the stereostructures of two unique stereoisomers of disinomenines with a C−C coupled frame of two sinomenine moieties and discovered that the oxidative dimerization products of sinomenine by $KMnO_4$ in an aqueous solution were stereoselsctively controlled by different pH values. The interesting thing is that (*S*)-disinomenine (**2**), a metabolite biocatalyzed only by *A. semisupina*, showed a more potent inhibitory activity on IL-6 production compared with that of sinomenine, while (*R*)-disinomenine (**3**), a chemically synthesized product, displayed a stimulative effect on IL-6 production. To explain the difference of the bioassay results of **2** and **3**, detailed research at the molecular level is essential. Further studies on the mechanism of pH-dependent, stereoselective coupling of sinomenine and expansion of dimers of sinomenine derivatives are now in progress.

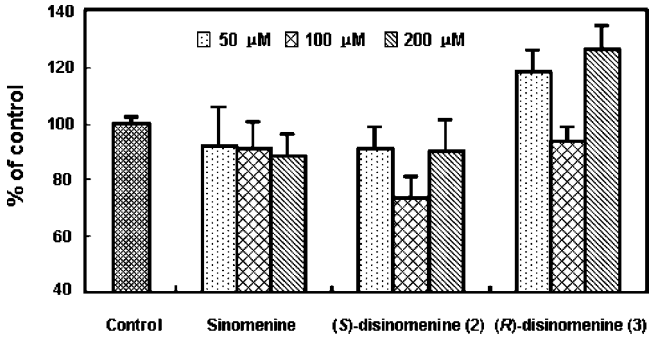

Figure 3. Inhibitory effects of sinomenine and (*S*)- and (*R*)-disinomenine (**2** and **3**) on IL-6 production in SW982 cells. Control: no added compounds; sinomenine, (*S*)- and (*R*)-disinomenine: added each compound, respectively. SW982 cells were cultured for 2 d with 1 ng/mL of IL-1β in the presence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

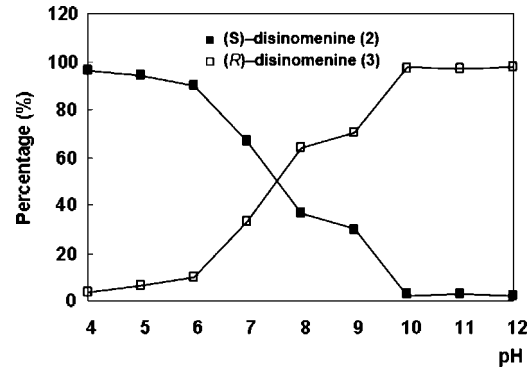

Figure 4. Oxidative dimerization of sinomenine by potassium permanganate at different pH values. Percent was obtained by HPLC analysis based on calibration curves of **2** and **3**.

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Supporting Information Available: Detailed descriptions of experimental procedures, full spectroscopic data of (*R*)-disinomenine, and bioactivity for related compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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