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NOTE

Isoquinoline alkaloids from *Zanthoxylum simulans* and their biological evaluation

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Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease characterized by synovial inflammation, destruction of joint cartilage and bone erosion, it affected 0.5 to 1% of the population.[1–4] At the present, the treatment of RA often involves the extensive use of disease-modifying anti-rheumatic drugs and anti-rheumatic biological drugs, such as methotrexate, salazosulfapyridine, iguratimod, tofacitinib and so on.[5–7] However, more attention has been focused on traditional Chinese medicine by their efficacy against RA and safety, for example, sinomenine that is extracted from *Sinomenium acutum* has shown therapeutic efficacy and few side effects in patients with RA in China since the 1990s.[8,9] Natural products have provided unlimited opportunities for new drug discoveries because of their inherent chemical diversity, and have prompted a continuous research for plant sources with medicinal value. *Zanthoxylum simulans* belongs to the genus *Zanthoxylum* of Rutaceae family. The barks are commonly used for the treatment of RA and swelling with unknown reasons in traditional Chinese medicine.[10] Previous chemical investigations on this genus have led to the identification of a series of alkaloids, coumarins, amides, lignans and flavonoids.[11–15] However, the specific components responsible for the therapeutic effects of this traditional Chinese medicine are still not well clarified. In our ongoing study to seek bioactive constituents from this traditional Chinese medicine against RA, we isolated a series of isoquinoline alkaloids, including three new alkaloids, zanthoxylumines A–C (**1**–**3**), together with six known compounds (Figure 1), 6-acetonyl-*N*-methyldihydrodecarine (**4**),[16] 8-hydroxy-7-methoxy-5-methyl-2,3-meth-ylenedioxybenzo[*c*] phenanthridin-6(5*H*)-one (**5**),[17] decarine (**6**),[18] norchelerythrine (**7**),[18] liriodenine (**8**)[19] and lysicamine (**9**).[19] Their structures were elucidated using extensive spectroscopic techniques. The isolated compounds were evaluated for their inhibitory activities against rat joint synovial cell proliferation, splenocyte proliferation and antimicrobial activities. We present herein the extraction, isolation, structure elucidation and biological activities of compounds **1**–**9**.

Compound **1** was obtained as white solid, and had a molecular formula $C_{19}H_{15}NO_4$ from the high resolution electron ionization mass spectra (HREIMS) (*m/z* 321.0992 $[M]^+$, calcd 321.1001). The ^1H and ^{13}C NMR data (Table 1) showed the characteristic pattern of a benzophenanthridine alkaloid.[16–18] The ^1H NMR spectrum exhibited signals at δ_H 7.56 (d, $J=8.9$ Hz), 8.40 (d, $J=8.9$ Hz), 8.41 (d, $J=8.9$ Hz), 7.84 (d, $J=8.9$ Hz), 7.31 (s), 8.62 (s) and 9.58 (s), indicating the presence of four aromatic hydrogens in *ortho* position and three isolated hydrogens, and it can be confirmed by the cross-peaks between H-9 (δ_H 7.56) and H-10 (δ_H 8.40), H-11 (δ_H 8.41) and H-12 (δ_H 7.84) in the 1H–1H COSY spectrum. Detailed analysis of the one-dimensional and two-dimensional NMR spectra data revealed that the NMR data of **1** were very similar to those of decarine (**6**),[18] except for the signals for the methylenedioxide group in decarine (**6**) were replaced by those for one methoxy group and one hydroxyl group in compound **1**. The methoxy group was assigned by the correlation from 3-OCH_3 to C-3 in the HMBC spectrum (Figure 2). The ROESY spectrum showed the correlations between protons H-12 and H-1/H-11, H-9 and H-10 (Figure 2). Therefore, compound **1** was elucidated as 2,8-dihydroxy-3,7-dimethoxybenzo[*c*]phenanthridine and named zanthoxylumine A.

Compound **2** was isolated as white solid. Its molecular formula was determined as $C_{20}H_{17}NO_4$ on the basis of the HREIMS (*m/z* 335.1155 $[M]^+$, calcd 335.1158). The ^1H and ^{13}C NMR data (Table 1) indicated that the structure of **2** was closely similar to that of **1**, differing in one more *O*-methyl in the structure of **2**, which was also supported by the correlation from 2-OCH_3 to C-2 in the HMBC spectrum (Figure 2). The ROESY spectrum showed the correlations between protons H-12 and H-1/H-11, H-9 and H-10 (Figure 2). Thus, compound **2** was

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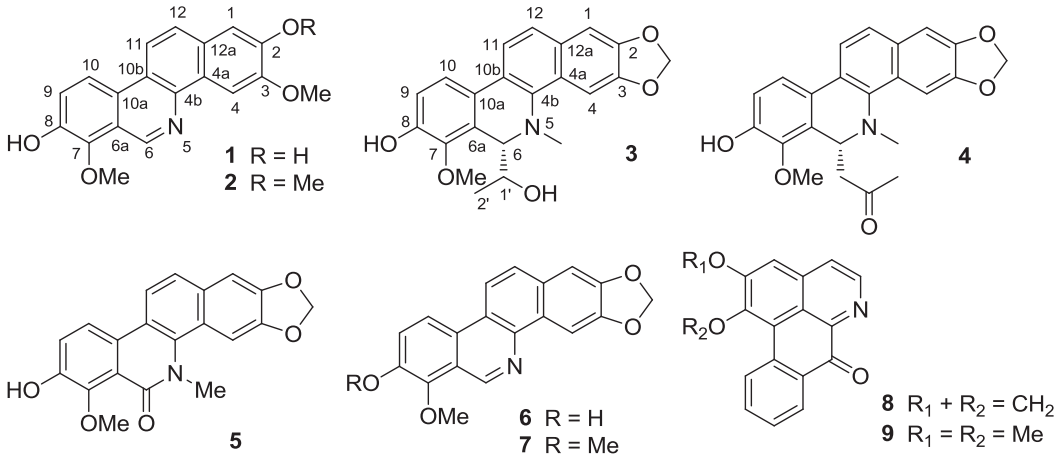

Figure 1 Chemical structures of compounds **1**–**9**.

Table 1 ^{1}H and ^{13}C NMR Data of compounds 1–3 (600 and 150 MHz, respectively, δ in p.p.m.)

	1[a]		*2*[b]		*3*[b]	
No.	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	112.0, CH	7.31, s	107.5, CH	7.53, s	104.2, CH	7.28, s
2	149.0, C		149.7, C		147.1	
3	150.8, C		149.6, C		147.9	
4	104.8, CH	8.62, s	103.4, CH	8.61, s	100.4, CH	7.81, s
4a	127.8, C		126.6, C		126.4	
4b	140.4, C		138.3, C		138.2	
6	146.7, CH	9.58, s	145.7, CH	9.59, s	64.1, CH	4.06, d (8.9)
6a	123.1, C		121.7, C		125.3	
7	143.9, C		142.1, C		145.5	
8	148.8, C		148.2, C		150.1	
9	124.9, CH	7.56, d (8.9)	123.9, CH	7.59, d (8.9)	116.4, CH	6.91, d (8.5)
10	119.9, CH	8.40, d (8.9)	118.7, CH	8.45, d (8.9)	119.1, CH	7.45, d (8.5)
10a	129.2, C		126.2, C		122.5	
10b	121.6, C		119.8, C		123.8	
11	119.2, CH	8.41, d (8.9)	118.4, CH	8.50, d (8.9)	119.6, CH	7.73, d (8.6)
12	128.1, CH	7.84, d (8.9)	126.7, CH	7.98, d (8.9)	123.6, CH	7.50, d (8.6)
12a	130.5, C		127.8, C		130.3	
2-OMe			55.6, CH_3	3.95, s		
3-OMe	56.5, CH_3	4.14, s	55.5, CH_3	4.01, s		
7-OMe	62.3, CH_3	4.10, s	61.1, CH_3	4.02, s	60.0, CH_3	3.82, s
OCH_2O					101.2, CH_2	6.13, s
N-Me					42.2, CH_3	2.58, s
1′					66.3, CH	3.04, m
2′					19.4, CH_3	0.89, d (6.3)

[a]Measured in CD_3OD.
[b]Measured in dimethyl sulfoxide-d_6.

elucidated as 8-hydroxy-2,3,7-trimethoxybenzo[*c*]phenanthridine and named zanthoxylumine B.

Compound **3** was obtained as white solid, gave an HREIMS peak at *m/z* 379.1423 $[M]^+$, corresponding to the molecular formula $C_{22}H_{21}NO_5$. The ^{1}H and ^{13}C NMR data (Table 1) also implied the characteristic pattern of a benzophenanthridine alkaloid as compounds **1** and **2**. Comparison of all NMR data with those of 6-acetonyl-*N*-methyldihydrodecarine (**4**) whic h was recently isolated from *Z. riedelianum*,[16] revealed a high degree of similarity. However, the only difference between them was the presence of 1-hydroxyethyl moiety instead of one acetonyl group. The 1-hydroxyethyl was located at C-6 by the cross-peaks between H-1′ (δ_H 3.04) and H-6 (δ_H 4.06)/H_3-2′ (δ_H 0.89) in the 1H-1H COSY spectrum and the correlation of δ_H 0.89 (H_3-2′)/C-6 in the HMBC spectrum (Figure 2). The ROESY spectrum showed the key correlations of H-6 with H-1′, H_3-2′ and 5-NCH_3 (Figure 2), consistent with 6-acetonyl-*N*-methyldihydrodecarine (**4**).[16] Consequently, compound **3** was determined as 6-(1-hydroxyethyl)-*N*-methyldihydrodecarine and named zanthoxylumine C.

All the isolated compounds were evaluated for their inhibitory activities against rat joint synovial cells proliferation, splenocytes proliferation and antimicrobial activities, respectively. Among them,

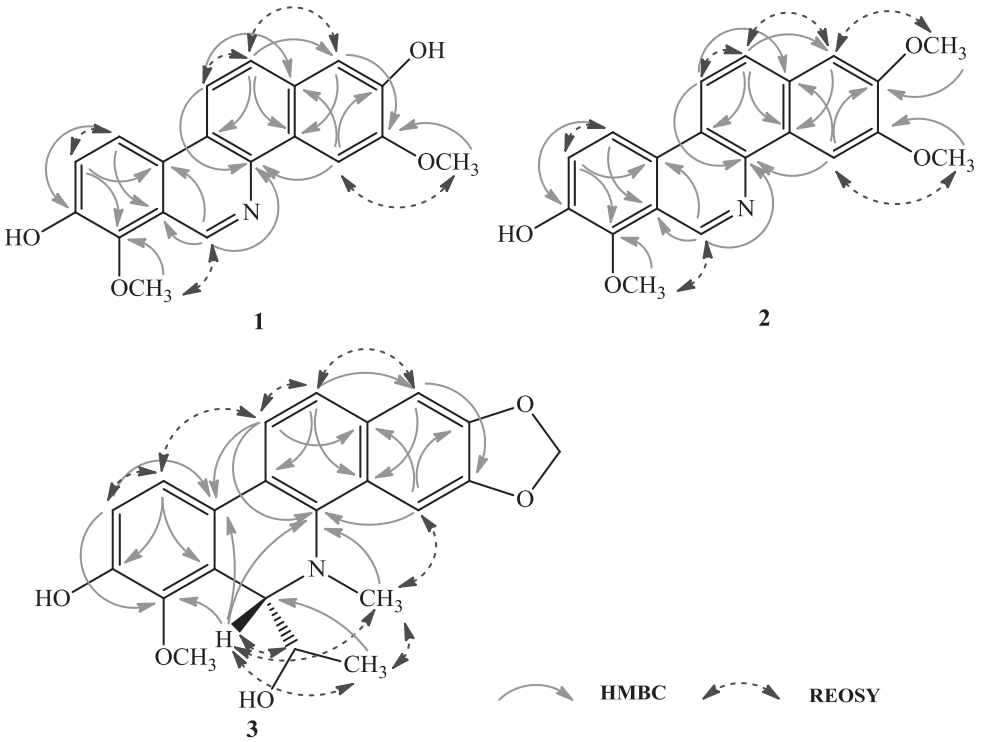

Figure 2 The key HMBC and ROESY correlations of **1**–**3.** A full color version of this figure is available at *The Journal of Antibiotics* journal online.

Table 2 Inhibitory activities of compounds 1–9 (IC_{50}, μM)

Compound	*Rat joint synovial cells*	*Splenocytes*
1	25.0	57.7
2	32.2	49.7
3	>50	39.1
4	38.1	>60
5	>50	>60
6	24.2	>60
7	35.3	>60
8	35.6	22.2
9	25.3	>60
Sinomenine	16.9	22.6

compounds **1**, **2**, **4** and **6**–**9** exhibited inhibitory activities against rat joint synovial cells proliferation with IC_{50} values ranging from 24.2 to 38.1 μM, and compounds **1**–**3** and **8** exhibited inhibitory activities against splenocytes proliferation with IC_{50} values ranging from 22.2 to 57.7 μM, respectively (Table 2). In addition, compounds **1** and **4** showed modest antimicrobial activities against *Enterococcus faecalis* with MIC values of 23.4 and 12.8 μM, respectively. This study not only provides a scientific rationale between the biological activity and the herbal remedies of this plant but also discloses compounds **1**–**4** and **6**–**9**, which may serve as potential drugs against RA for future clinical treatment.

MATERIALS AND METHODS

General experimental procedures

Optical rotations were recorded on a Horiba SEPA-300 polarimeter. UV spectra were measured on a Shimadzu UV-2401PC spectrophotometer (Shimadzu Corporation, Kyoto, Japan). IR spectra were obtained on a Tensor 27 (Bruker Optics Gmbh, Ettlingen, Germany) with KBr pellets. NMR spectra were recorded on the Bruker AV-400, DRX 500 and Bruker Avance III-600 MHz spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany). EI-MS was obtained on a Finnigan-4510 spectrometer. ESI-MS and HREIMS were determined with an API QSTAR Pulsar 1 spectrometer (MDS Sciex, Concord, ON, Canada). Silica gel (200–300 mesh, Qingdao Marine Chemical Qingdao, China), RP-18 gel (40–63 μm, Daiso, Osaka, Japan), and Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden) were used for column chromatography. Semi-preparative HPLC (SHIMADZU LC-10A HPLC system (Shimadzu Corporation), Ultimate XB-C-18, 5 μm, 10 × 250 mm).

Plant materials

The barks of *Z. simulans* (30 kg) were purchased from Hunan Corporation of Materia Medica, Hunan Province, China, in February 2011, and authenticated by corresponding author (XJZ). A voucher specimen (ZHXJ-0012) was deposited at our laboratory in Hunan University of Chinese Medicine.

Extraction and isolation

The dried powdered bark of *Z. simulans* (30 kg) was extracted with ethanol (2 × 180 l) to yield an extract (3370 g), which was suspended in water and partitioned by petroleum ether and EtOAc (each 4 × 8 l). The EtOAc extract (470 g) was fractionated by a silica gel column eluted with $CHCl_3$ with increasing amounts of methanol to yield seven fractions (Frs 1–7). Fr 2 (89 g) was divided into five parts (Frs 2-1–2-5) by an MCI gel CHP 20 P column eluting with gradient aqueous MeOH. Fr 2-3 (9.5 g) chromatographed by silica gel column (petroleum ether/EtOAc, 20:1, 15:1, 10:1, 5:1, 2:1, 1:1) to separate three parts (Frs 2-3-1–2-3-3), compound **1** (9.3 mg) was purified by RP-18 gel column (MeOH-H_2O 1:1–1:0), silica gel column (petroleum ether/EtOAc, 4:1) and Sephadex LH-20 (MeOH) from Fr 2-3-3 (1.8 g). Fr 2-3-1 (1.4 g) first submitted by RP-18 gel column (MeOH-H_2O 1:1–1:0), followed by Sephadex LH-20 (MeOH) and further purified by semi-preparative HPLC (SHIMADZU LC-10A HPLC system, Ultimate XB-C-18, 5 μm, 10 × 250 mm) eluting with 75% aqueous MeOH to yield compound **6** (10.5 mg) and compound **7**

(12.8 mg). Fr 2-4 (11.1 g) was chromatographed by vaccum liquid chromatography on a silica gel column eluted with petroleum ether/EtOAc (10:1, 5:1, 10:1, 4:1, 3:1, 2:1, 1:1) to divide into four parts (Frs 2-4-1–2-4-4). Frs 2-4-1 (2.9 g) was first chromatographed by RP-18 gel column (MeOH-H_2O 1:1–1:0), followed by Sephadex LH-20 (MeOH), and finally purified by semi-preparative HPLC (64% aqueous MeOH) to yield compound **2** (8.1 mg) and compound **3** (14.0 mg). Fr 2-4-2 (1.3 g) was separated by RP-18 gel column (MeOH-H_2O 1:1–1:0) and followed by semi-preparative HPLC (70% aqueous MeOH) to yield compound **4** (17.5 mg) and compound **5** (7.2 mg). Fr 2-2 (7.2 g) was first submitted to silica gel column (petroleum ether/EtOAc, 30:1, 25:1, 20:1, 15:1, 10:1, 5:1, 1:1), followed by Sephadex LH-20 (MeOH) and RP-18 gel column (MeOH-H_2O 4:6–1:0) to produce compound **8** (6.3 mg) and compound **9** (45.1 mg).

Zanthoxylumine A (**1**). White solid; UV (MeOH) λ_{max} (log ε) 274.5 (2.85), 252.0 (2.83) and 202.0 (2.78) nm; IR (KBr) ν_{max} 3441, 1630, 1464, 1456 and 1384 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EIMS *m/z* 321 $[M]^+$; HREIMS 321.0992 $[M]^+$ (calcd for $C_{19}H_{15}NO_4$, 321.1001).

Zanthoxylumine B (**2**). White solid; UV (MeOH) λ_{max} (log ε) 274.0 (3.95) and 206.5 (3.84) nm; IR (KBr) ν_{max} 3441, 1626, 1462, 1438 and 1384 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EIMS *m/z* 335 $[M]^+$; HREIMS 335.1155 $[M]^+$ (calcd for $C_{20}H_{17}NO_4$, 335.1158).

Zanthoxylumine C (**3**). White solid; $[\alpha]^{15.4}_D$—7.2 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) = 282.4 (4.08) and 227.2 (4.01) nm; IR (KBr) ν_{max} 3448, 1635, 1486, 1423 and 1384 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS *m/z* = 380 $[M+H]^+$; HREIMS 379.1423 $[M]^+$ (calcd. for $C_{22}H_{21}NO_5$, 379.1420).

One-dimensional and Two-dimensional NMR and HREIMS spectra of compounds **1–3** and bioassay protocols are available in Supplementary Information.

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Supplementary Information accompanies the paper on The Journal of Antibiotics website (http://www.nature.com/ja)