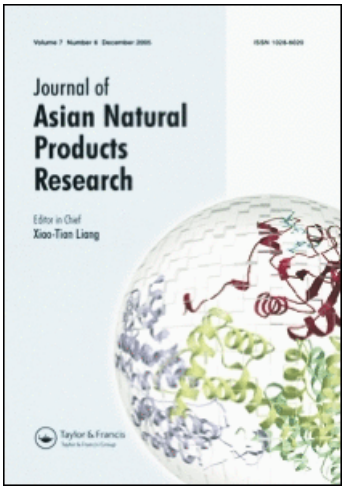

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Three major urinary metabolites of sinomenine in rats

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Three major urinary metabolites of sinomenine in rats

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Urinary metabolites of sinomenine were investigated in rats after intragastric administration. Three major metabolites were obtained and characterised as 4-hydroxy-3,7,7-trimethoxy-17-methyl-(9α,13α,14α)-morphinan-6-one (**1**), 7,8-didehydro-4-hydroxy-3,7-dimethoxy-17-methyl-*N*-oxide-(9α,13α,14α)-morphinan-6-one (**2**), and 7,8-didehydro-4-hydroxy-3,7-dimethoxy-(9α,13α,14α)-morphinan-6-one (**3**). Their structures have been elucidated on the base of spectral analysis, among which **1** and **2** were new compounds.

Keywords: Sinomenine; Metabolite; Rat; Urine

1. Introduction

Sinomenine is the main anti-inflammatory constituent of *Sinomenium acutum*, and its hydrochloric salt has been used clinically for the treatment of rheumatoid arthritis in China for many years [1–3]. It was also reported to possess other pharmacological effects [4–8], such as immunosuppression, consciousness sedation, anti-arrhythmia, analgesia, protection of organs from damage caused by shock and Ca-antagonism. However, little information is available on the metabolism of sinomenine, though its pharmacokinetic works have been much reported [9–12]. Our studies on the metabolism of sinomenine led to the identification of three major metabolites in the urine of rats. The present paper describes the isolation and the structural elucidation of the metabolites (figure 1).

2. Results and discussion

Metabolite **1**, obtained as white amorphous powder, was positive to Dragendorff's reagent, and exhibited absorption at 259 nm in the UV spectrum. The positive ESI-MS spectrum of **1** showed a quasi-molecular ion peak $[M + H]^+$ at *m/z* 362, and $[M + H{-}CH_3O]^+$ at *m/z* 332. The positive HR ESI-MS spectrum showed a quasi-molecular ion peak $[M + H]^+$ at *m/z*

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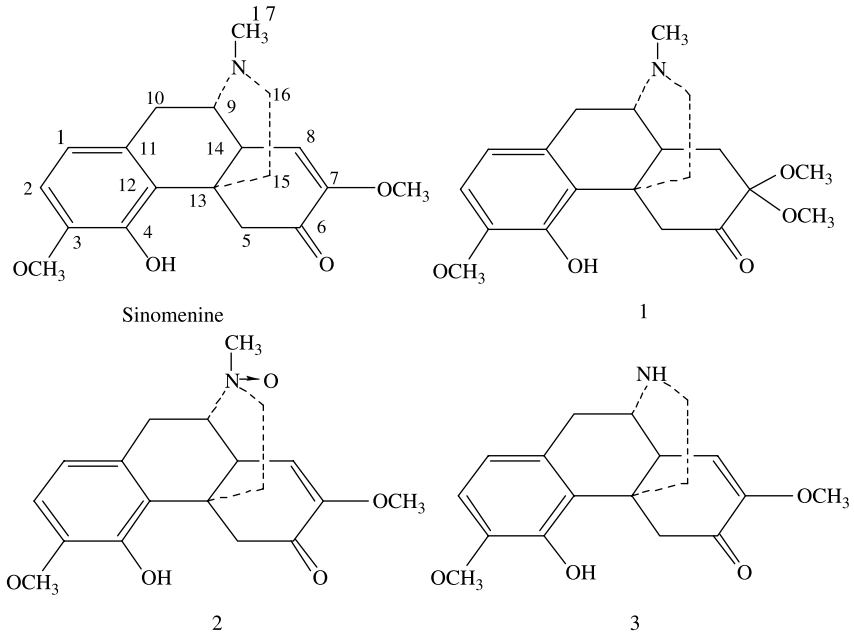

Figure 1. The structures of sinomenine and metabolites **1, 2** and **3**.

362.1892 (calcd for $C_{20}H_{27}NO_5$, 362.1877); combined with the ^{1}H NMR and ^{13}C NMR spectral data, the molecular formula of **1** was determined to be $C_{20}H_{27}NO_5$. Comparison of the ^{13}C NMR spectrum of **1** with those of sinomenine showed that there are close similarities except for the signals of C-5–C-8: the double-bond carbon signals at δ 154.0 (C-7) and 113.4 (C-8) of sinomenine disappeared and the two new corresponding carbon signals at δ 101.4 and 37.1 were observed in **1**. Meanwhile, the carbonyl signal shifted downfield from δ 194.7 (C-6) to 207.1, and δ 47.3 (C-5) shifted upfield to δ 38.4 in **1**. These changes indicated the α,β-conjugated double bond had been saturated. In the HMBC spectrum (figure 2), δ 3.19 (7-OCH_3), 3.15 (7-OCH_3), δ 2.25 (H-8, dd, $J = 3.4$, 13.4 Hz) and 1.46 (H-8, dt, $J = 1.1$, 13.4 Hz) correlated with δ 101.4 (C-7), δ 2.25 (H-8) with δ 207.1 (C-6), δ 2.25 (H-8) and 1.46

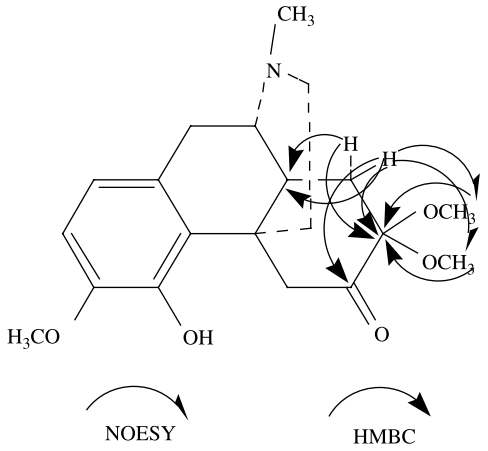

Figure 2. The key HMBC and NOESY correlations of **1**.

(H-8) with δ 42.6 (C-14), and δ 2.55 (H-5) with δ 207.1 (C-6), 123.1 (C-12), 42.7 (C-13) and 38.4 (C-15). Additionally, δ 2.44 (H-8) had correlations with the two methoxyl groups at δ 3.19 (7-OCH_3) and 3.15 (7-OCH_3) in the NOESY spectrum. The above evidence established the variations that occurred in the structure of **1** compared with that of sinomenine.

Thus, the structure of metabolite **1** was identified as 4-hydroxy-3,7,7-trimethoxy-17-methyl-(9α,13α,14α)-morphinan-6-one. The ^{1}H NMR and ^{13}C NMR data of metabolite **1** were fully assigned on the basis of 2D NMR techniques, including HMQC, ^{1}H–^{1}H COSY and HMBC (tables 1 and 2).

Metabolite **2**, obtained as white amorphous powder, was positive to Dragendorff's reagent, and exhibited absorption at 262 nm in UV spectrum. The positive ESI-MS spectrum of **2** showed a quasi-molecular ion peak $[M + H]^+$ at *m/z* 346, 16 mass units higher than that of sinomenine. The positive HRESI-MS spectrum showed a quasi-molecular ion peak $[M + H]^+$ at *m/z* 346.2315 (calcd for $C_{19}H_{23}NO_5$, 346.2327); combined with the ^{1}H NMR and ^{13}C NMR spectral data, the molecular formula of **2** was determined to be $C_{19}H_{23}NO_5$. Comparison of the ^{13}C NMR spectrum of **2** with those of sinomenine showed that there are close similarities except for the signals of the carbons around the N atom: C-9, − 16 and − 17 at the α-position of the N atom in **2** shifted downfield by 15.8, 13.5 and 17.1 ppm, respectively, and C-10, − 14 and − 15 at the β-carbons of the N atom shifted upfield by 4.3, 2.3 and 6.1 ppm, respectively. In the ^{1}H–^{1}H COSY spectrum, δ 3.27 (H-17) showed correlations with δ 3.70 (H-9) and δ 3.10 (H-16), δ 3.70 (H-9) with δ 3.19 (H-10), and δ 3.10 (H-16) with δ 2.44 (H-15). The above evidence indicated that **2** remained the skeleton of sinomenine and the additional oxygen atom should be linked to the N atom. Therefore, **2** was identified as sinomenine-*N*-oxide. The formation of *N*-oxide is a common metabolic pathway. The full assignments of carbon and proton signals were assigned on the basis of HMBC, ^{1}H–^{1}H COSY and NOESY spectra, and are summarised in tables 1 and 2.

Table 1. ^{13}C NMR data of sinomenine and metabolites **1, 2** and **3**.

No.	*Carbon signals*			
	Sinomenine	***1***	***2***	***3***
1	118.8	119.5	119.7	119.6
2	110.1	111.1	111.7	111.3
3	146.1	147.3	148.0	147.6
3-OCH_3	56.2	56.6	56.6	56.6
4	145.0	146.8	146.9	146.8
5	47.3	38.4	49.3	49.2
6	194.7	207.1	195.4	195.6
7	154.0	101.4	153.8	153.8
7-OCH_3	55.1	49.1	55.5	55.4
7-OCH_3	–	49.8	–	–
8	113.4	37.1	115.4	115.4
9	58.5	57.9	74.3	51.3
10	25.1	24.6	29.4	31.0
11	125.4	131.1	127.0	129.7
12	120.4	123.1	122.1	122.8
13	38.9	42.7	40.5	41.6
14	42.7	42.6	40.5	45.0
15	38.4	38.4	32.5	35.0
16	48.3	47.6	61.8	39.4
17	41.1	42.5	58.2	–

Recorded on a Bruker ARX 400 instrument, in CD_3OD. The carbon and proton signals were assigned unambiguously on ^{1}H NMR, ^{13}C NMR, COSY, NOESY, HMQC and HMBC.

Table 2. ^{1}H NMR data of sinomenine and metabolites **1, 2** and **3**.

No.	*Proton signals*			
	Sinomenine	***1***	***2***	***3***
1	6.68	6.57	6.59	6.58
2	6.85	6.74	6.80	6.78
3	–	–	–	–
3-OCH_3	3.82	3.79	3.79	3.78
4	–	–	–	–
5	4.44, 2.61	3.34, 2.55	4.40, 2.54	4.38, 2.51
6	–	–	–	–
7	–	–	–	–
7-OCH_3	3.51	3.19	3.48	3.49
7-OCH_3	–	3.15	–	–
8	5.77	2.25, 1.46	5.71	5.72
9	3.82	2.98	3.70	3.76
10	3.32, 3.24	2.97, 2.69	3.34, 3.19	3.34, 2.88
11	–	–	–	–
12	–	–	–	–
13	–	–	–	–
14	3.37	2.41	4.05	3.06
15	2.15	1.91, 1.76	2.44, 1.89	2.02, 1.86
16	3.29, 2.71	2.46, 2.03	3.10, 2.93	2.93, 2.60
17	2.99	2.38	3.27	–

Recorded on a Bruker ARX 400, in CD_3OD. The carbon and proton signals were assigned unambiguously on ^{1}H NMR, ^{13}C NMR, COSY, NOESY, HMQC and HMBC.

Metabolite **3**, obtained as white amorphous powder, was positive to Dragendorff's reagent, and exhibited absorption at 264 nm in the UV spectrum. The positive ESI-MS spectrum showed a quasi-molecular ion peak $[M + H]^+$ at *m/z* 316, and $[M + Na]^+$ at 338, 14 mass units lower than that of sinomenine. Combined with the ^{1}H NMR and ^{13}C NMR spectral data, the molecular formula of **3** was determined to be $C_{18}H_{21}NO_4$. Comparison of the ^{13}C NMR data of **3** with those of sinomenine showed that there are similarities in the sinomenine skeleton except for the signals of the carbons around the N atom. C-9 and C-16 attributable to α-carbons of the N atom shifted upfield by 7.2 and 8.9 ppm, respectively, and C-10 and C-14 ascribable to the β-carbons of the N atom shifted downfield by 5.9 and 2.3 ppm, respectively. In the ^{1}H–^{1}H COSY spectrum, δ 3.76 (H-9) showed correlations with δ 2.88 (H-10) and δ 3.06 (H-14), and δ 2.93 (H-16) with δ 1.86 (H-15) and δ 2.44 (H-15). In the HMBC spectrum, δ 5.72 (H-8) showed correlations with δ 153.8 (C-7) and δ 45.0 (C-14), and δ 3.34 (H-10) showed correlations with δ 129.7 (C-11), 122.8 (C-12) and 119.6 (C-1). The above evidence indicated that **3** kept the parent structure of sinomenine. Compared with ref. [14], **3** was identified as *N*-demethylsinomenine. The full assignments of carbon and proton signals were based on the HMBC, ^{1}H–^{1}H COSY and NOESY spectra, and are summarised in tables 1 and 2.

3. Experimental

3.1 General experimental procedures

3.1.1 Chemicals. Sinomenine, with a purity of 99.0% in HPLC, was purchased from Xi'an Sanxin Bio-pharmaceutical Company. For column chromatography, silica gel (200–300 mesh), silica gel G_{60} and GF_{254}, and ODS were commercially obtained from the Chemical

Plant of Nankai University and Pharmacia, respectively. Sephadex LH-20 was the product of Pharmacia. Preparative HPLC was performed using a C-8 column (C-8, 250 × 20 mm, Inertsil Pak; detector: UV). All the mobile phase solvents, analytical grade for open column chromatography and chromatographic grade for HPLC, were purchased from the Chemical Solvent Company of Shenyang.

3.1.2 Instruments. UV spectra were recorded on a Shimadzu UV-2200 Ultraviolet Spectrophotometer. NMR spectra were recorded on an ARX 400 spectrometer. Chemical shifts were obtained as δ value (ppm) downfield relative to tetramethylsilane. ESI-MS spectra were measured with an LC-MSD-Trap-SL mass spectrometer. The preparative HPLC system for purification of metabolites consisted of an Alltech 426 HPLC pump and Waters 490 programmable Multiwavelength Detector.

3.2 Experimental animals

Male Wistar rats (200–250 g body weight, 8–10 weeks old) were provided by the Animal Centre of Shenyang Pharmaceutical University. These animals were kept in a breeding room to be acclimatised for 7 days before use. Normal food and water were available at all times but withdrawn 24 h prior to intragastric administration of sinomenine.

3.3 Isolation of metabolites from rat urine

Rat urine (about 360,000 ml) was collected using the metabolic cage after intragastric administration to rats of sinomenine at a dose of 40 mg/kg. The total amount of sinomenine administered was about 15 g, and the whole period of administration lasted about 3 months. With methanol added, the urine sample was then successfully concentrated under 45°C and vacuum conditions. After extraction with chloroform, the metabolites were enriched in the chloroform layer, which were further chromatographed on a silica column. The fragment eluted with $CHCl_3$/MeOH (7:3) was then subjected to an ODS column, followed by preparative HPLC (column: Xterrra ODS; flow rate: 10 ml/min; detection wavelength: 259, 262 and 264 nm, respectively) to yield 24 mg of **1**, 20 mg of **2** and 13 mg of **3**.

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References

[1] K.K. Zhang, C.E. Ding, X. Zhou. *Hunan J.T.C.M.*, **12**, 9 (1998).
[2] J.B. Liu, X.C. Chen. *J. Xi'an Med. Univ.*, **19**, 391 (1998).
[3] C.F. Li, S.Q. Tong, W. Huang. *Capital M.*, **9**, 62 (2002).
[4] Y. Wang, Y. Fang. *J. Ethnopharmacol.*, **98**, 37 (2005).
[5] L. Liu, X.J. Li, L.X. Wang. *Zhongguo Mianyixue Zazhi*, **18**, 241 (2002).
[6] H.R. Huo, X.P. Che. *J. Xi'an Medical Univ.*, **10**, 346 (1989).
[7] Z.S. Liu, Q. Yang. *J. Beijing Coll. T.C.M.*, **10**, 41 (1989).

[8] Z.Y. Lao. *Chin. J. New. Drug Clin. Rem.*, **19**, 254 (2000).
[9] T.H. Tsai, J.W. Wu. *Life Sci.*, **72**, 2413 (2003).
[10] S.D. Luo, X.G. Pan. *Zhongyaocai*, **14**, 36 (1991).
[11] X.H. Zhou, H.D. Li, W.X. Peng. *Acta Pharm. Sin.*, **32**, 620 (1998).
[12] Q.E. Liu, M.R. Liang. *Zhongyao xinyao yu linchaung yaoli*, **6**, 23 (1995).
[13] T. Yoshihilo, T. Kazuo, M. Shin. *Tetrahedron Lett.*, **33**, 2853 (1975).
[14] C. Uwe, V.W. Kaever. *PCT Int. Appl. WO*, **99**(42), 105.