Asian Journal of Chemistry; Vol. 24, No. 11 (2012), 5222-5224

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

Antibacterial Evaluation of Piperidine Alkaloids from *Cassia siamea* Lam

XINZHOU YANG[1], PING ZHAO[2], XINQIAO LIU[1] and ZHINAN MEI[1,*]

[1]College of Pharmacy, South-Central University for Nationalities, 708 Min-Zu Road, Wuhan 430074, P.R. China
[2]College of Life Sciences, South-Central University for Nationalities, 708 Min-Zu Road, Wuhan 430074, P.R. China

*Corresponding author: Fax: +86 27 67843220; Tel: +86 27 67841196; E-mail: yxzyxz01@163.com

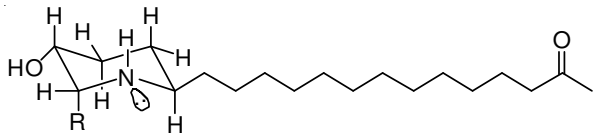
(*Received*: 10 October 2011; *Accepted*: 15 June 2012) AJC-11601

Bioassay directed isolation of the EtOAc extract from a chinese folk herb *Cassia siamea* Lam resulted in the purification of a new piperidine alkaloid (-)-(2R,3R,6R)-7-hydroxyspectaline (**1**), as well as a known analogue, (-)-(2R,3R,6R)-*iso*-6-spectaline (**2**). The structure of **1** was determined by extensive 1D and 2D NMR and MS data analyses. Compound **1** was isolated during a drug discovery program aimed at identifying new antibacterial leads from a prefractionated natural product library. Antibacterial study showed that both compounds are potent antimicrobial agents with MIC values range of 12.5-50 μg/mL against methicillin-resistant *Staphylococcus aureus* and *S. epidermidis*, respectively.

Key Words: *Cassia siamea* Lam, Piperidine alkaloids, Antimicrobial activity.

INTRODUCTION

Bacterial infections are common illness, which can lead to serious, even life-threatening complications, such as sepsis, kidney failure, toxic shock syndrome and death. Especially amongst hospitalized patients and those with chronic diseases, such as heart disease, diabetes, AIDs and cancer, are at high risk of serious bacterial infection[1]. Natural products have played a very important role in antibiotic drug discovery[2]. Well known examples include penicillin G from *Penicillium chrysogenum* and erythromycin from *Streptomyces erythreus*, which were discovered and commercialized in 1940s and 1950s, respectively[3]. Since then, a significant number of NP-derived antibacterial compounds have undergone clinical evaluations[2]. However, most antibiotics are losing effectiveness against pathogenic microorganisms and showing cross-resistance to many current drugs, including penicillins[4]. Additionally, Gram-negative pathogens are becoming increasingly resistant to antibiotics[5]. Therefore, there is an urgent need for new lead compounds to feed into the drug development pipeline in the search for new and effective antibiotics to fight drug-resistant bacterial. In a screening of 400 traditional Chinese medicines as well as Chinese folk herbal plants against methicillin-resistant *Staphylococcus aureus* (MRSA) and *S. epidermidis* (MRSE) isolated from Huashan Hospital in Shanghai, an EtOAc extract of the Chinese folk herb Tiedaomu (Leaves of *Cassia siamea* Lam, Fabaceae) displayed promising activity. Bioassay-guided fractionation on the large-scale organic extract resulted in the isolation of a new piperidine alkaloid, (-)-(2R,3R,6R)-7-hydroxyspectaline (**1**), as well as a known analogue, (-)-(2R,3R,6R)-*iso*-6-spectaline (**2**) (Fig. 1). Herein we report the isolation, structure elucidation for **1** and antibacterial activity for compounds **1** and **2**.



1 R=CH_2OH
2 R=CH_3

Fig. 1. Structures for compounds **1** and **2**

EXPERIMENTAL

The leaves of *Cassia siamea* Lam (Caesalpiniaceae) were collected during July of 2006 at Jinxiu County, Guangxi province, China and and identified by Prof. Chaoliang Zhang of Guangxi Botanical Garden of Medicinal Plants. A voucher specimen (No. YA00012) has been deposited in the Herbarium of South-Central University for Nationalities, Wuhan, P.R. China.

Extraction and isolation: Air-dried leaves of *Cassia siamea* Lam (100 g) were ground and then extracted by maceration at room temperature with *n*-hexane (3 × 1.0 L, 4 h each), followed by ethyl acetate (3 × 1.0 L, 4 h each) and methanol (3 × 1.0 L, 4 h each). The solvents were evaporated at reduced pressure to yield 1.9, 3.6 and 5.3 g of *n*-hexane,

TABLE-1
SPECTROSCOPIC DATA FOR THREE TYPES OF PIPERIDINE ALKALOIDS

	$[\alpha]_D$	H-2	H-3	H-6	C-2	C-3	C-6	Reference
1[a]	-6.8 (MeOH)	3.16 dd, 6.5, 3.1	3.64 br.s	3.01 m	58.4	64.7	55.8	–
2[a]	-6.5 (MeOH)	3.14 dd, 6.7, 2.7	3.72 br.s	2.97 m	56.8	63.2	55.6	–
[a]	-7.0 ($CHCl_3$)	2.98 br d, 6.5, 2.5	3.66 br.s	3.02 ddd, 7.2, 5.5, 5.0	58.3	66.6	57.6	[7]
3[a]	-6.0 ($CHCl_3$)	3.21 dd, 6.5, 2.6	3.81 br.s	3.02 m	58.5	66.0	57.3	[8]
4[b]	-3.6 (MeOH)	3.28 dd, 6.5, 2.8	3.82 br.s	3.02 m	58.5	66.0	57.5	[8]
5[a]	-8.2 ($CHCl_3$)	2.72 dq, 6.5, 1.2	3.57 br.s	2.50 m	55.7	67.9	57.2	[8]
	-12.0 (CH_2Cl_2)							[9]
6[a]	-7.0 (CH_2Cl_2)	2.70 dq, 7.5, 2.0	3.76 br.s	2.52 m	61.1	66.2	56.9	[8]
7[a]	-8.5 ($CHCl_3$)	2.72 dq, 6.5, 1.2	3.57 br.s	2.48 m	55.7	68.0	57.2	[8,10]
8[a]	+8.0 ($CHCl_3$)	2.9, m	3.55 br.s	2.70 m	57.0	67.6	55.7	[9]
9	[c]	2.76, m	3.55 s	2.54 m	57.4	68.1	56.0	[11,12]
10[a]	+2.5 ($CHCl_3$)	2.72 dq, 6.5, 1.2	3.53, br.s	2.51, m	55.8	68.0	56.6	[8]

[a]NMR data in $CDCl_3$; [b]NMR data in CD_3OD; [c]cassine was always obtained as racemic; **2**: iso-6-spectaline; **3**: iso-6-canavaline; **4**: leptophyllin B; **5**: (-)-spectaline; **6**: (-)-7-hydroxyspectaline; **7**: (-)-spectalinine; **8**: spectaline; **9**: (±) cassine; **10**: leptophyllin A

ethyl acetate and methanol extract, respectively. 3.2 g of the EtOAc fraction was subjected to CC over polyamide resin (200 g) eluted with 0, 30, 50, 70 and 95 % aq. EtOH in a step manner. The 70 % EtOH fraction (1.2 g) was subjected to preparative HPLC with the YMC-Pack Diol 5 µm (20 mm × 150 mm i.d.) HPLC column (MeOH in CH_2Cl_2 from 10 % to 40 %, 120 min) to yield **1** (1.4 mg, tR 69.2 min) and **2** (3.4 mg, tR 84.7 min), respectively. The purity of the isolated two compounds was above 95 % (HPLC).

(-)-(2R,3R,6R)-*iso*-7-hydroxyspectaline (1): Light yellow oil; $[\alpha]_D^{25}$ -6.8 (0.1, CH_3OH), -6.1 (0.1, $CHCl_3$); IR ν_{max} (film) 3743, 3392, 1685, 1640, 1501, 1371, 1202, 983 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz): δ_H 3.70 (1H, dd, *J* = 11.2, 6.5 Hz, H-7), 3.68 (1H, dd, *J* = 11.2, 4.6, H-7), 3.64 (1H, br.s, H-3), 3.16 (1H, dd, *J* = 6.5, 3.1 Hz, H-2), 3.01 (1H, m, H-6), 2.38 (2H, t, *J* = 7.3 Hz, H-12'), 2.04 (3H, s, H-14'), 1.79 (1H, dd, *J* = 13.4, 10.2 Hz, H-4α), 1.60 (1H, m, H-4β), 1.45 (2H, m, H-1'), 1.42 (2H, m, H-11'), 1.29 (2H, m, H-5), 1.24-1.26 (18H, br.s, H-4' - H-10'), 1.23-1.24 (4H, m, H-2'-H-3'); ^{13}C NMR ($CDCl_3$, 150 MHz): 209.8 (C-13'), 64.7 (C-3), 58.4 (C-2), 57.6 (C-7), 55.8 (C-6), 40.1 (C-12'), 33.3 (C-1'), 30.2 (C-14'), 29.8 (C-4), 29.7-30.1 (C-4'-C-10'), 29.5 (C-3'), 26.9 (C-2'), 25.4 (C-5), 23.7 (C-11'); HRESIMS *m/z* 342.3012 $[M+H]^+$ ($C_{18}H_{16}NO_3$, calcd. 342.3008).

Antimicrobial assay: It was determined by the broth dilution technique as previously described[6]. The solutions (maximum concentration) of the compounds (*i.e.* the compounds that induced zones of inhibition) were prepared in DMSO, serially (2-fold) diluted and 0.5 mL of each dilution was introduced into a test tube containing 4.4 mL of selenite broth; then 0.1 mL of microbial suspension (5×10^5 cfu/mL) was added and the mixture was homogenized. The total volume of the mixture was 5 mL, with the test compound concentrations in the tube ranging from 100 to 6.25 µg/mL and those of the standard compounds, *i.e.* vancomycin, ranging from 10 to 0.625 µg/mL, respectively. After 24 h of incubation at 37 ºC, the MIC was reported as the lowest concentration of a compound that prevented visible growth.

RESULTS AND DISCUSSION

An air-dried sample of the plant *Cassia siamea* Lam (100 g) was extracted successively with *n*-hexane, EtOAc and MeOH. The EtOAc extract (3.2 g) was subjected to repeated column chromatography of normal phase silica gel and preparative normal phase HPLC with a diol semi-preparative HPLC column to afford (-)-(2R, 3R, 6R)-7-hydroxyspectaline (**1**, 1.4 mg, 0.028 % dry wt.), as well as a known analogue, (-)-(2R, 3R, 6R)-*iso*-6-spectaline (**2**, 3.4 mg, 0.068 % dry wt).

Compound **2** was determined to be (-)-(2R,3R,6R)-*iso*-6-spectaline following spectroscopic data comparison with literature values[6].

Compound **1**, (-)-(2R,3R,6R)-*iso*-6-spectaline, was isolated as optically active light yellow powder. The (+)-LRESIMS of **1** showed a strong $[M+H]^+$ peak at *m/z* 342. HRESIMS measurement on $[M+H]^+$ ion (*m/z* 342.3012), in combination with ^1H and ^{13}C (from HSQC and HMBC) spectroscopic data (Table-1), supported the molecular formula of $C_{20}H_{39}NO_3$ with two double bond equivalents. Its IR spectrum showed strong absorption bands for a hydroxyl group (3424 cm^{-1}), secondary amine (3341, 1550 cm^{-1}) and ketone functional groups (1715 cm^{-1}).

The joint analysis of the ^1H NMR and HSQC spectra of **1** showed one hydroxymethine at δ_H 3.64 (br.s, H-3), two methines at δ_H 3.16 (1H, dd, *J* = 6.5, 3.1 Hz, H-2) and 3.01 (1H, m, H-6), an oxygenated methylene at δ_H 3.70 (1H, dd, *J* = 11.2, 6.5 Hz, H-7), 3.68 (1H, dd, *J* = 11.2, 4.6, H-7), a methylene at δ_H 2.38 (2H, t, *J* = 7.3 Hz, H-12'), a methyl group at δ_H 2.04 (3H, s, H-14'), a methylene at δ_H 1.79 (1H, dd, *J* = 13.4, 10.2 Hz, H-4α) and 1.60 (1H, m, H-4β) and three multiplet methylenes at δ_H 1.45, 1.42 and 1.29, as well as several methylene protons at δ_H 1.24-1.26 (H-4'-H-10'). The ^{13}C NMR spectrum showed the presence of three methine carbons at δ_C 64.7, 58.4 and 55.8, a methylene carbon at δ_C 57.6, a ketone carbon at δ_C 209.8, a methyl carbon at δ_C 21.3 and several methylene carbons at δ_C 29.7-30.1. Comparison of the ^1H and ^{13}C NMR spectra of **1** with those of (2R, 3R, 6R)-*iso*-6-spectaline (**2**), strongly suggested **1** was very similar to **2** except for the data related to the piperidine ring. The ^{13}C NMR spectrum of **1** showed the same resonances as **2** with exception of the values for C-2 (58.4), C-3 (64.7), C-6 (55.8) and C-7 (57.6), indicating that a hydroxymethylene at C-7 replaced a methyl group in **1**. The significant shielding observed for C-3 (64.7) compared to that in **2** could be explained by the g-gauch effect of the hydroxymethylene at C-7. The detailed analysis

of the long range HMBC correlations approved to assign all ^{13}C resonances (Fig. 2). The relative configurations of the chiral carbons C-2, C-3 and C-6 in the piperidine ring of **1** were established by comparison of the ^{1}H NMR data observed for **1** with those published for **2** and ROESY correlations (Table-1 and Fig. 2).

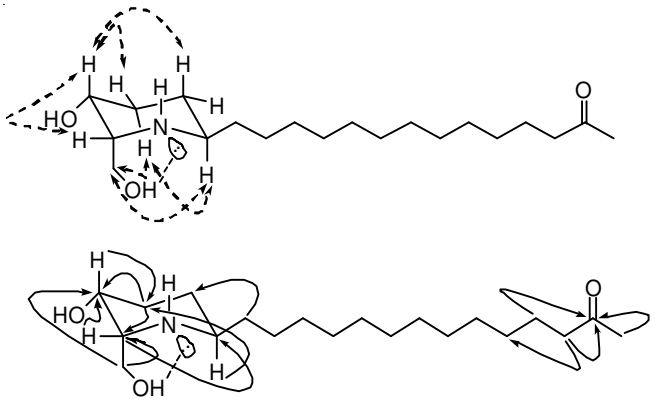
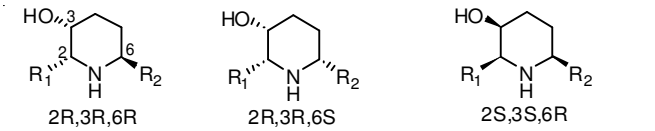


Fig. 2. Key ROESY correlations (⟵ - - ⟶) and HMBC correlations (⟶) for compound **1**

Piperidine alkaloids are abundant in natural, especially occurring in Cassia or Prosopis species and many of them, especially those with 2,6-dialkylpiperidine 3-ol system showed interesting pharmaceutical properties. Up to now, about 60 samples of 2,6-dialkylpiperidine 3-ol alkaloids have been isolated, showing DNA-damaging activity[7,8], acetylcholinesterase[13], lipoperoxidation and cyclooxygenase enzyme inhibition[14], as well as antinociceptive activity[15]. Based on the differences of three chiral carbons C-2, C-3 and C-6 in the piperidine ring, these alkaloids can be classified into three types, namely 2R,3R,6S,2R,3R,6R and 2S,3S,6R (Fig. 3)[8]. The spectroscopic properties of several representatives belonging to three types of alkaloids were summarized in Table-1. The $[\alpha]_D$ value for **1** was determined to be - 6.80 and the NMR data for three chiral centers were assigned as: H-2 [3.16 dd (6.5, 3.1)], C-2 (58.4); H-3 (3.64 br.s), C-3 (66.5); H-6 (3.01, m), C-6 (55.8). All the above data was consistent with those of 2R,3R,6R piperidine alkaloids[8]. Therefore, the structure of **1** was suggested to be (-)-(2R,3R,6R)-*iso*-7-hydroxyspectaline.



2R,3R,6R

1 R_1=CH_2OH, R_2=$(CH_2)_{12}COCH_3$
2 R_1=CH_3, R_2=$(CH_2)_{12}COCH_3$
3 R1=CH_3, R_2=$(CH_2)_{10}CH(OH)CH_3$
4 R1=CH_3, R_2=$(CH_2)_{10}CO_2H$

2R,3R,6S

5 R_1=CH_3, R_2=$(CH_2)_{12}COCH_3$
6 R_1=CH_2OH, R_2=$(CH_2)_{12}COCH_3$
7 R1=CH_3, R_2=$(CH_2)_{12}CH(OH)CH_3$

2S,3S,6R

8 R_1=CH_3, R_2=$(CH_2)_{12}COCH_3$
9 R_1=CH_3, R_2=$(CH_2)_{10}COCH_3$
10 R1=CH_3, R_2=$(CH_2)_{10}CH(OH)CH_2OH$

Fig. 3. Three types of piperidine alkaloids based on three chiral centres

The antimicrobail activity of **1** and **2** was evaluated *in vitro* against MRSA and MRSE (Table-2). Compounds **1** and **2** exhibited potent activity against MRSA and MRSE with MIC values range of 12.5-50 μg/mL.

TABLE-2
ANTI-MICROBIAL ACTIVITY OF COMPOUNDS **1** AND **2** AGAINST MRSA AND MRSE IN MIC VALUES (μg/mL)

	MRSA	MRSE
1	25	50
2	12.5	25
Vancomycin[a]	0.625	1.25

[a]Vancomycin was used as positive control

(-)-(2R,3R,6R)-7-hydroxyspectaline (**1**) and (-)-(2R,3R,6R)-*iso*-6-spectaline (**2**) are the representatives of a new scaffold of antibacterial agents. The discovery of a new scaffold for antibacterial agents widens the spectrum of natural products acting on this target. The example of piperidine alkaloids highlights the advantages of our lead-like discovery approach based on prefractionated library and bioassay-directed isolation.

ACKNOWLEDGEMENTS

The work was financially supported by the Talented Faculty Foundation of South-Central University for Nationalities (YZ211002 to X.Z. Yang) and National Natural Science Foundation of China (31070744 to P. Zhao).

REFERENCES

1. L. Vitetta, D. Kenner and A. Sali, *J. Pain Sympt. Manag.*, **20**, 326 (2000).
2. M.S. Butler, *Nat. Prod. Rep.*, **25**, 475 (2008).
3. D.J. Christensen, E.B. Gottlin, R.E. Benson and P.T. Hamilton, *Drug Discov. Today*, **6**, 721 (2001).
4. K. Hiramatsu, *Am. J. Med.*, **104**, 7S (1998).
5. S.J. Wadsworth, K.H. Kim, V. Satishchandran, P. Axelrod, A.L. Truant and B. Suh, *J. Antimicrob. Chemother.*, **30**, 821 (1992).
6. A.F. Barrero, J.F. Moral, A. Lara and M.M. Herrado, *Planta Med.*, **71**, 67 (2005).
7. C. Virgas Jr., V. da S. Bolzami, M. Furlan, E.J. Barreiro, M.C.M. Young, D. Tomazela and M.N. Eberlin, *J. Nat. Prod.*, **67**, 908 (2004).
8. V. da S. Bolzani, A.A.L. Gunatilaka and D.G.I. Kingston, *Tetrahedron*, **51**, 5929 (1995).
9. I. Christofiedis, A. Welter and J. Jadot, *Tetrahedron*, **33**, 977 (1977).
10. I. Christofiedis, A. Welter and J. Jadot, *Tetrahedron*, **33**, 3005 (1977).
11. E. Schu and A. Bonte, *Tetrahedron Lett.*, **16**, 2881 (1975).
12. C.A. Leverett, M.P. Cassidy and A. Padwa, *J. Org. Chem.*, **71**, 8591 (2006).
13. C. Jr. Virgas, V.S. Bolzami, L.S.B. Pimentel, N.G. Castro, R.F. Cabral, R.S. Costa, C. Floyd, M.S. Rocha, M.C.M. Young, E.J. Barreiro and C.A.M. Fraga, *Bioorg. Med. Chem.*, **13**, 4184 (2005).
14. C. Jr. Virgas, D.H.S. Silva, M. Pivatto, A. Rezende, I. Castro-Gamboa, V.S. Bolzami and M.G. Nair, *J. Nat. Prod.*, **70**, 2026 (2007).
15. C. Jr. Virgas, M.S. Alexandre-Moreira, C.A.M. Fraga, E.J. Barreiro, V.S. Bolzami and A.L.P. Miranda, *Chem. Pharm. Bull.*, **56**, 407 (2008).