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Alkaloids from the seeds of *Sterculia lychnophora* (Pangdahai)

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Abstract

Two alkaloids, named sterculinine I and sterculinine II, together with thirteen known compounds were isolated from the ethanol extract of a well-known Chinese traditional drug, Pangdahai (the seeds of *Sterculia lychnophora* Hance). Their structures were elucidated by NMR, UV, IR and MS spectroscopic analysis.

Keywords: *Sterculia lychnophora*; Sterculiaceae; Alkaloid; Quinolinone; Aspartate

1. Introduction

Pangdahai (Boat-fruited Sterculia Seed) is a traditional Chinese drug and specified as the seeds of *Sterculia lychnophora* Hance in the Chinese pharmacopoeia (The Pharmacopoeia Commission of PRC, 2000). This traditional drug is reputed for its prevention of, and as a remedy against, pharyngitis. It has also been used for the treatment of tussis and constipation since ancient times in China. The original plant, *S. lychnophora* (Sterculiaceae) is distributed in VietNam, Thailand, Malaysia, Indonesia as well as the southeastern part of China. Though flavonoids, terpenoids, phenolics, and histamines were reported to exist in other species of genus *Sterculia* (Ranganathan and Nagarajan, 1980; Anjaneyulu and Raju, 1987; Hayman et al., 1988), no specific compounds except for polysaccharides and fatty acids were isolated from Pangdahai (Chen et al., 1995). The present paper deals with the isolation and structure determination of two new alkaloids, named sterculinine I (**1**) and sterculinine II (**2**) from the ethanol extract of this traditional drug. In addition, 13 known compounds, soya-cerebroside II (**3**), 1-*O*-β-D-glucopyranosyl-(*2S*,*3R*,*4E*,*8Z*)-2-[(2-hydroxy-icosanoyl)amido]-4,8-octadecadiene-1,3-diol (**4**), kaempferol-3-*O*-β-D-glucoside (**5**), uracil (**6**), succinic acid (**7**), 2, 4-dihydroxy benzoic acid (**8**), daucosterol (**9**), β-sitosterol (**10**), sucrose (**11**), isorhamnetin-3-*O*-β-D-rutinoside (**12**), kaempferol- 3-*O*-β-D-rutinoside (**13**), *n*-butyl-α-D-mannopyranside (**14**), and adenosine (**15**) were obtained from the extract. Compounds **3**–**7** and **12**–**15** were also isolated from this traditional drug for the first time.

2. Results and discussion

The ethanol extract of Pangdahai was subjected to partition and column chromatography to afford compounds **1**–**9** from the ethyl acetate (EtOAc)-soluble part, compound **10** from the cyclohexane-soluble part, and compounds **11**–**15** from the *n*-butanol-soluble part, respectively.

Compound (**1**) displayed the molecular formula $C_{18}H_{20}N_2O_6$ from its HR-ESI-MS, indicating 10 degrees of unsaturation. The UV spectrum of **1** disclosed α,β-unsaturated carbonyl and benzyl chromophores at 331 and 271 nm, respectively. Its IR spectrum exhibited amide, carbonyl and aromatic absorption bands at 3299, 1735, 1641, 1600, 1464, 754 and 655 cm^{-1}. The ^1H NMR spectrum (Table 1) showed resonances for five aromatic proton signals, 12 aliphatic proton signals and three proton signals on heteroatoms. The combination of ^{13}C NMR and DEPT experiments revealed the existence of one methyl, four methylenes, six methines, and seven quaternary carbons.

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Table 1
^1H and ^{13}C NMR spectral data for **1** and **2** (in DMSO-d_6)

Position	**1**		**2**	
	^1H[a] δ, J in Hz	^{13}C[b] δ	^1H[a] δ, J in Hz	^{13}C[b] δ
1	11.96 (1H,*brs*)	–	11.93 (1H,*brs*)	–
2	–	161.1	–	161.3
3	6.44 (1H,*brs*)	119.8	6.45 (1H,*brs*)	119.6
4	–	145.8	–	145.9
5	7.73 (1H,*d*,7.0)	125.9	7.74 (1H,*d*,7.0)	125.9
6	7.20 (1H,*m*)	122.0	7.20 (1H,*m*)	122.2
7	7.55 (1H,*m*)	131.0	7.53 (1H,*m*)	131.0
8	7.35 (1H,*d*,8.0)	115.6	7.34 (1H,*d*,8.0)	115.6
9	–	139.2	–	139.1
10	–	116.0	–	116.1
1′	–	165.7	–	165.8
2′	9.24 (1H,*d*,8.0)	–	9.18 (1H,*d*,8.0)	–
3′	4.81 (1H,*m*)	49.1	4.78 (1H,*m*)	49.0
4′a	2.87 (1H,*dd*,17.0,6.0)	35.5	2.67 (2H,*m*)	36.3
4′b	2.76 (1H,*dd*,17.0,8.0)	35.5	–	–
5′	–	170.5	–	170.9
6′	4.12 (2H,*t*,6.5)	64.7	3.67 (3H,s)	52.3
7′	1.60 (2H,*m*)	30.1	Not observed	171.5
8′	1.36 (2H,*m*)	18.5		
9′	0.89 (3H,*m*)	13.5		
10′	12.35 (1H,s)	171.4		

[a] 500 MHz.
[b] 125 MHz.

Three moieties (Fig. 1) could be established according to the spectroscopic data from ^1H–^1H COSY and HMQC of **1**. Four methine groups [δ_H 7.73 (1H, *d*, J = 7.0 Hz, H-5), 7.55 (1H, m, H-7), 7.35 (1H, *d*, J = 8.0 Hz, H-8) and 7.20 (1H, m, H-6); δ_C 125.9 (C-5), 131.0 (C-7), 115.6 (C-8) and 122.0 (C-6)] built into moiety I in an aromatic ring. A methylene group [δ_H 2.87 (1H, *dd*, J = 17.0 and 6.0 Hz, H-4′) and 2.76 (1H, *dd*, J = 17.0 and 8.0 Hz, H-4′); δ_C 35.5 (C-4′)], a methine group [δ_H 4.81(1H, m, H-3′); δ_C 49.1(C-3′)], and an amide proton [δ 9.24 (1H, *d*, J = 8.0 Hz, H-2′)] formed moiety II. Moiety III, as a butyl group, comprised one methyl group [δ_H 0.89 (3H, *m*, H_3-9′); δ_C 13.5 (C-9′)] and three methylene groups [δ_H 4.12 (2H, *t*, J = 6.5 Hz, H_2-6′), 1.60 (2H, *m*, H_2-7′) and 1.36 (2H, *m*, H_2-8′); δ_C 64.7 (C-6′), 30.1 (C-7′) and 18.5 (C-8′)]. The three moieties and the remaining signals could be assembled together on the basis of the evidence from HMBC (Fig. 2). The correlations between H-7 or H-5 and C-9 (δ 139.2) and between H-6 or H-8 and C-10 (δ 116.0) suggested the presence of a disubstituted benzene ring. HMBC correlations observed between H-5 and C-4 (δ 145.8) and between H-3 [δ 6.44 (1H, *br* s)] and C-10 enabled the connection of C-4 to C-10 and C-3 to C-4. Similarly, HMBC correlations between H-3 and C-1′ (δ 165.7) and between H-3′ or H-2′ and C-1′ gave evidence an aliphatic chain connected to C-4 through an amide group. The proton signal at δ 11.96 (H-1) should be a NH substituent of the disubstituent benzene ring, because it correlated with C-10 and C-3. Meanwhile, the molecular formula revealed that there were two nitrogen atoms in the molecule. The remaining carbonyl group C-2 (δ 161.1) should be between the NH and C-3, considering the correlation between H-1 and C-3, the downfield chemical shift of the NH proton, and the degrees of unsaturation of this compound. Thus, the quinolin-2-one ring was deduced and both the ^1H and ^{13}C NMR spectral data were in agreement with the skeleton (Brown et al., 1980). The aliphatic chain was composed of moiety II and moiety III. Moiety II joined C-4 through C-1′ as already mentioned above. Moiety III connected with C-4′ through an ester group, where both H-3′ and H-6′ were found to correlate with C-5′ (δ 170.5). Major correlation was also observed between H-4′ and C-10′ (δ 171.4), which gave evidence that there is a carboxyl substituent at C-3′ in **1**. The TOF-MS/MS of **1** displayed a base peak at m/z 172 due to the cleavage of the peptide bond between C-1′ and N-2′ in **1**. The quinolin-2-one ion peak was observed at m/z 144 when cleavage occurred between C-4 and C-1′. A fragment peak corresponding to the loss of the butoxy groups (m/z 287) from **1** was also observed in the MS spectrum. On the basis of the above evidence, **1** was 2-[(2-oxo-1,2-dihydro-quinoline-4-carbonyl)-amino]-succinic acid 4-*n*-butyl ester.

Compound **2** is an analog of **1**. The HR-ESI-MS data of **2** gave the molecular formula $C_{15}H_{14}N_2O_6$. The UV

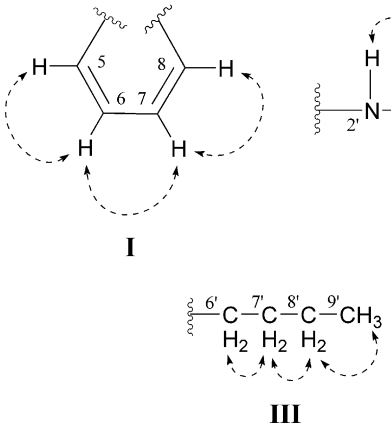

Fig. 1. Three moieties and ^1H-^1H COSY correlations of **1**.

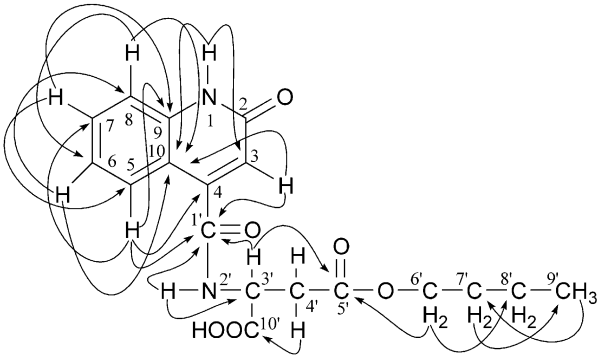

Fig. 2. Important HMBC correlations of **1**.

spectrum of **2** also revealed α,β-unsaturated carbonyl and benzyl chromophores at 333 and 275 nm, respectively. Its IR spectrum demonstrated amide, carbonyl and aromatic absorption bands at 3273, 1738, 1646, 1543, 1464, 756 and 656 cm^{-1}. The ^1H and ^{13}C NMR spectral data of **2** were basically similar to those of **1,** except that the spectral data of **2** lack the three methylene group signals in **1** and the methyl group signal [δ_H 3.67 (3H, *s*, H_3-6′); δ_C 52.3 (C-6′)] in **2** was distinctly oxygenated (Table 1). Considering that **2** had the same degree of insaturation as **1**, it could be calculated that **2** had a methyl group in place of the butyl group at C-6′ in **1**. The structure of **2** was established by careful comparison of its ^1H and ^{13}C NMR spectral signals with those of **1** and by detailed 2D NMR spectroscopic analysis, including application of ^1H–^1H COSY, HMQC and HMBC experiments. The methyl group in **2** was confirmed to connect with C-4′ through an ester group by HMBC experiment (Fig. 3), which showed the correlation between H-6′ and C-5′ (δ 170.9). Therefore, **2** was 2-[(2-oxo-1,2-dihydro-quinoline-4-carbonyl)-amino]-succinic acid 4-methyl ester.

1 and **2** were confirmed to be natural products by HPLC analysis of the EtOAc-soluble components of an EtOH extract of the crude drug, which were performed in the absence of methanol and *n*-butanol. **1** and **2** are the first examples of natural products containing 2-oxo-1,2-dihydroquinoline-4-carboxylic acid core from Sterculiaceae. This type of compound was only reported in a few families such as Simaroubaceae and Poaceae (Tateishi et al., 1987; Yu and Li, 1990; Chung and Woo, 2001).

Known compounds **3**–**15** were characterized by comparing their physical and spectroscopic data with literature values (Jung et al., 1976; Markham et al., 1976; Shibuya et al., 1990; Zhao et al., 1991; Si et al., 1993; Chen et al., 1995).

3. Experimental

3.1. General

Melting points were determined on an XT-4A micro-melting point apparatus without correction. The IR spectra were recorded on a Thermo Nicolet Nexus 470 FT-IR spectrometer. UV spectra were measured on a Varian Cary Eclipse 300 spectrometer using H_2O as the solvent. Optical rotations were measured with an AA-10R polarimeter manufactured by Optical Activity Co. Ltd. Both ^1H and ^{13}C NMR experiments were performed on a Bruker DRX 500 NMR spectrometer using solvents as internal standards. The HR-ESI-MS spectra were detected with a Bruker APEX II mass spectrometer and TOF-MS/MS spectra were obtained with a PE Q-STAR ESI-TOF-MS/MS spectrometer. HPLC was performed on an Agilent 1100 HPLC apparatus equipped with G1315A DAD detector, G1312A Bin-Pump and HP Chemstation software. CC was carried out with silica gel (200–300 mesh) provided by Tsingtao Marine Chemistry Co. Ltd., Sephadex LH-20 (18–110 μm) manufactured by Pharmacia Co. Ltd., and ODS (100–200 mesh) made by Fuji Silysia Chemical Co. Ltd.

3.2. Plant material

Pangdahai was purchased in April, 2001 from the Anguo Chinese crude drug market in Hebei Province of China; Prof. Shao-Qing Cai authenticated them as the seeds of *S. lychnophora* Hance. Voucher specimens (No. 2592) were deposited in the herbarium of Pharmacognosy, School of Pharmaceutical Sciences, Peking University.

3.3. Extraction and isolation

Powdered Pangdahai (18 kg) was extracted with EtOH–H_2O (95:5, 90 l) under reflux for 3 h. Concentration of the EtOH solution in vacuo afforded a crude EtOH extract (1.9 kg). The crude ethanol extract was suspended in water and partitioned successively with cyclohexane, EtOAc, and *n*-butanol. The EtOAc-soluble part (80 g) was subjected to silica gel CC using $CHCl_3$–MeOH (30:1–3:1) as the eluent to afford 10 fractions (fractions A–J). Fraction D (10 g) was further applied to a silica gel column eluted with gradient (20:1–2:1) of $CHCl_3$–MeOH to give five sub-fractions (sub-fractions 1–5). Sub-fraction 3 (2.5 g) of fraction D was purified with Sephadex LH-20 CC using MeOH as eluent, to yield **2** (10 mg). Fraction G (7.5 g) was subdivided into several sub-fractions using the silica gel column eluted with gradient $CHCl_3$–MeOH (30:1–2:1). The sub-fraction 2 (98 mg) of fraction G was subjected to repeated silica gel CC using gradient $CHCl_3$–MeOH (20:1–2:1) as eluent to obtain crude **1**, which was subsequently purified by Sephadex LH-20 chromotograph (with MeOH as eluent) to yield **1** (8 mg). The other fractions of the EtOAc-soluble part were treated with repeated chromatography, including silica gel CC,

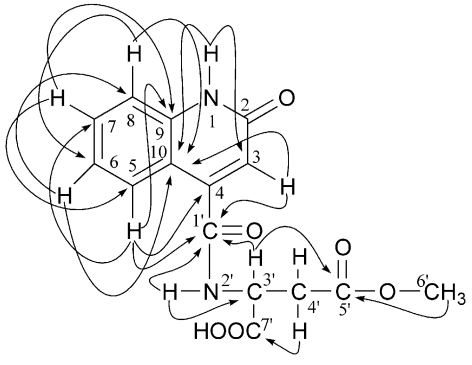

Fig. 3. Important HMBC correlations of **2**.

Sephadex LH-20 CC and prep TLC, to obtain **3** (20 mg), **4** (18 mg), **5** (12 mg), **6** (350 mg), **7** (187 mg), **8** (210 mg) and **9** (1230 mg). **11** (27 mg), **12** (35 mg), **13** (32 mg), **14** (56 mg) and **15** (62 mg) were obtained from the *n*-butanol-soluble part (180 g) after repeated CC on silica gel, Sephadex LH-20 and ODS. **10** (2580 mg) was isolated from the cyclohexane-soluble part (300 g) with repeated CC.

3.4. Confirmation of the existence of *1* and *2* in crude drug with HPLC

Powdered Pangdahai (100 g) was extracted with EtOH–H_2O (95:5, 400 ml) under reflux for 3 h. Concentration of the EtOH solution in vacuo yielded a crude EtOH extract (10.3 g). The crude ethanol extract was suspended in water and extracted successively with cyclohexane and EtOAc (80 ml). After evaporation of the EtOAc solution in vacuo, the residue (230 mg) was redissolved in 5 ml EtOH. From this sample solution, 40 μl was used for HPLC analysis. The HPLC conditions were as follows: column: RP-18 (4.6×250 mm, Waters, USA); eluent: CH_3CN–H_2O (10:90, v/v), 1 ml min^{-1} for 60 min; detector: G1315A DAD, λ 334 nm. The two peaks detected at Rt 14.209 min and 16.635 min showed the same retention time and UV spectra as for **1** and **2.**

3.5. 2-[(2-oxo-1,2-Dihydro-quinoline-4-carbonyl)-amino]-succinic acid 4-n-butyl ester (*1*)

White powder, mp 184–186 °C; $[\alpha]_D^{20}$ +24.2 (H_2O; *c* 0.50); UV λ_{max} (H_2O) nm (log ε): 331 (3.12), 271 (3.75); IR (KBr) cm^{-1}: 3419, 3299 (NH), 2960, 1735 (C=O), 1641, 1600, 1464, 1271, 1123, 872, 754, 655, 599; HR-ESI-MS (positive) *m/z*: 361.1397 $[M+1]^+$ (Calc. for $C_{18}H_{21}N_2O_6$: 361.1394); TOF-MS/MS (positive) *m/z*: 361 $[M+1]^+$, 305, 287 [M-butoxy]$^+$, 241, 215, 199, 187, 172 [quinolin-2-one-4-acyl]$^+$, 144 [quinolin-2-one skeleton]$^+$; ^{1}H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6), see Table 1.

3.6. 2-[(2-oxo-1,2-Dihydro-quinoline-4-carbonyl)-amino]-succinic acid 4-methyl ester (*2*)

White powder, mp 176–178 °C; $[\alpha]_D^{20}$ +25 (H_2O; *c* 0.50); UV λ_{max} (H_2O) nm (log ε): 333 (3.15), 275 (3.76); IR (KBr) cm^{-1}: 3273 (NH), 3068, 2962, 2854, 1738 (C=O), 1646, 1543, 1464, 1432, 1297, 1226, 756, 656, 556 cm^{-1}; HR-ESI-MS (positive) *m/z*: 319.0926 $[M+1]^+$ (calc. for $C_{15}H_{15}N_2O_6$: 319.0925); ^{1}H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6), see Table 1.

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