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Antibacterial steroidal alkaloids from *Holarrhena antidysenteriaca*

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[ABSTRACT] Two new steroidal alkaloids, isoconkuressine and *N*-formylconessimine, together with 6 known steroidal alkaloids including conkuressine, conessine, isoconessimine, conimine, conarrhimine, and funtudienine, were isolated from the seeds of *Holarrhena antidysenteriaca* Wall.ex A.DC. Their intrinsic antibacterial activities and synergistic effects with penicillin and vancomycin were analyzed in methicillin sensitive *staphylococcus aureus* (MSSA) and methicillin resistant *staphylococcus aureus* (MRSA). Two of the steroidal alkaloids including one new compound (*N*-formylconessimine) showed potential antibacterial activity and possessed synergistic effects with penicillin and vancomycin, respectively.

[KEY WORDS] *Holarrhena antidysenteriaca*; Steroidal alkaloid; Antibacterial; MRSA; MSSA; Synergistic effect

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Introduction

Holarrhena antidysenteriaca Wall.ex A.DC. also called "zhixiemu" in China, is mainly distributed in India, Thailand, Myanmar, and Southwestern China. It is often used for the treatment of diarrhea, intestinal parasites, constipation, and fever in folk medicines [1]. Previous studies have indicated that the extract of *H. antidysenteriaca* which contains alkaloids has antibacterial [2], antimalarial [3], antidiabetic [4], and acetylcholinesterase inhibitory activities [5], but the active constituents in *H. antidysenteriaca* are still unknown. The antibacterial tests in previous studies of the extract use mainly gram-negative bacteria, such as *Escherichia coli* and *Acinetobacter baumannii* [6], and the antibacterial active constituents against gram-positive bacteria, especially drug resistance strains, have not been reported.

In the present study, eight steroidal alkaloids, including two new compounds (**1**, **4**) and six known compounds (**2**−**3**, **5**−**8**), were isolated and their structures were identified. The antibacterial activities against *staphylococcus aureus* (MSSA) and *staphylococcus aureus* (MRSA) were performed. Additionally, their synergistic antibacterial effects with penicillin and vancomycin against MSSA and MRSA were also determined.

Results and Discussion

The 75% ethanol extract was dissolved in water and then partitioned with petroleum ether, ethyl acetate and butanol, respectively. Compound 4 was obtained in ethyl acetate fraction and compounds **1**−**3**, **5**−**8** were obtained in butanol fraction with several chromatography methods. The structures were elucidated as isoconkuressine (**1**), conkuressine (**2**), conessine (**3**), *N*-formylconessimine (**4**), isoconessimine (**5**), conimine (**6**), conarrhimine (**7**), and funtudienine (**8**), based on spectroscopic data. Among these 8 steroidal alkaloids, compounds **1** and **4** were new compounds.

Compound **1** was a white needle crystal in methanol, with a molecular formula of $C_{23}H_{38}N_2$ determined by HR-ESI-MS (*m/z* 343.311 4 calculated for $C_{23}H_{39}N_2$: 343.310 8). Based on the positive result of $KBiI_4$ reaction on TLC and the NMR data, compound **1** was suggested to be a steroidal alkaloid with an unsaturated bond in the skeleton. Besides C-2, C-3, C-4, other ^{13}C NMR data of compound **1** is similar to **3** which contains a β-CH_3 on C-10. Trans-fused B and C rings, C and D rings. Compound **3** was reported to contain in the

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bark of this plant [7-8]. The missing ^{1}H NMR signals of two methyl groups (δ 2.21, 6H, s) comparing with compound **3** suggested the presence of $NHCH_3$ group on C-3 in compound **1** (δ 2.30, 3H, s). In NOESY, H-3 (δ 2.25) had correlation with H-1β (δ 1.99) which had correlation with H-19 (δ 0.89). This result confirmed the α-$NHCH_3$ group on C-3. The β-CH_3 on C-20 was also confirmed by the correlation between H-21 (δ 1.00) and H-16β (δ 1.58). Based on the correlation between H-22 (δ 2.15) and H-21, a β-CH_3 on position 22 was identified, due to the formation of sp^3 type of the nitrogen atom in the E ring. Thus the structure of compound **1** was identified as isoconkuressine.

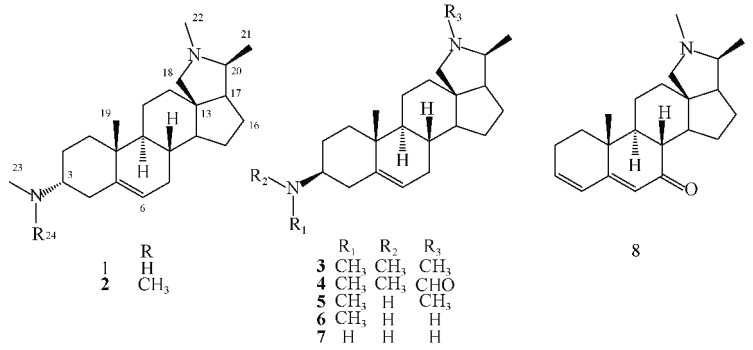

Fig. 1 Structures of 1−8

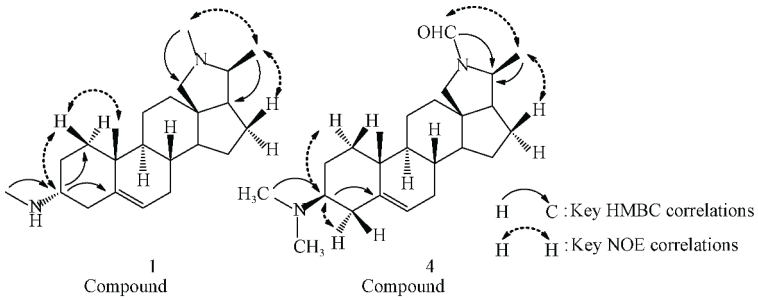

Fig. 2 Key HMBC and NOE correlations of 1 and 4

Compound **4** was a white powder in methanol, with a molecular formula of $C_{24}H_{38}N_2O$ determined by HR-ESI-MS (*m/z* 371.305 1, calculated for $C_{24}H_{39}N_2O$: 371.305 7). The similar NMR data with compound **3** suggested a same skeleton. Instead of ^{1}H NMR signal of CH_3-22 on compound **3**, the ^{1}H- (δ 8.08) and ^{13}C- (δ 162.7) NMR data of compound **4** suggested the presence of an aldehyde group on the nitrogen atom. In HMBC the correlation between aldehyde proton (H-22) and C-20 confirmed that the aldehyde group was connected on the nitrogen atom at position 22. In NOESY, the β-CH_3 on C-20 was confirmed by the correlation between H-21 (δ 1.32) and H-16β (δ 1.62), and the correlation between H-22 (δ 8.08) and H-21 (δ 1.32) identified the β-CHO on the nitrogen atom in the E ring. In NOESY, H-3 (δ 2.12) had correlation with H-1α (δ 1.47) and H-4α (δ 1.37) which suggested the presence of β-$N(CH_3)_2$ group on C-3. Thus the structure of compound **4** was identified as *N*-formylconessimine. The NMR data of compounds **1** and **4** are shown in Table 1.

The structures of other compounds were determined by comparing the NMR data with the references. Compounds **2**, **3**, **5**, **6**, **7** and **8** were identified as conkuressine, conessine, isoconessimine, conimine, conarrhimine, and funtudienine respectively [5, 7].

The intrinsic antibacterial activity test showed compounds **4** and **6** possessed some activities with MIC of 32 and 128 μg·mL^{-1}against both MSSA and MRSA, respectively, using penicillin and vancomycin as the positive controls. As shown in Table 2, in the synergistic antibacterial test, both Compounds **4** and **6** showed synergistic effect against MRSA and MSSA when combined with penicillin and vancomycin, respectively. Besides, compound **6** also possessed the synergistic effect against MRSA with vancomycin, while compound **4** only showed additional effect. Compounds **4** and **6** showed no relative effect with penicillin against MSSA. The synergistic effect was determined by fractional inhibitory concentration (FIC). Although the mechanism for synergistic antibacterial activity was unknown, it was indicated that they might be caused by weakening outer membrane of pathogen [8].

Experimental

General experimental procedures

Optical rotations were measured using an Anton Paar MCP200 polarimeter (Anton Paar, Austria). IR spectra were recorded using a Bruker IFS 55 spectrometer (Bruker, saarbrücken, Germany).