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Monoterpene indole alkaloids from *Rhazya stricta*

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ABSTRACT

Twenty-seven monoterpene indole alkaloids (MIAs) including three new ones were isolated from the plant of *Rhazya stricta*. Their structures were elucidated by analyses of HRMS and NMR data. Secopleiocarpamine A (**1**) represents a novel 2,3-*seco* pleiocarpamine type MIA possessing a cyano group. A possible biosynthetic pathway for **1** was postulated. All compounds were evaluated for their inhibitory activities against six *Candida* strains, and the results showed that **2**, **5**, **12**, **21**, **23**, and **27** exhibited moderate inhibitory activities with MIC values ranging from 3.125 to 50 μg/mL.

1. Introduction

The plant *Rhazya stricta* Decne. (Apocynaceae) is an evergreen shrub widely distributed in South Asia (Pakistan, India, and Afghanistan) and the Middle East (e.g. Saudi Arabia, Qatar, UAE, Iran, and Iraq) [1]. As an important folk medicinal plant, it has been used for the treatment of various diseases such as chronic rheumatism, fever, inflammation, diabetes, sore throat, skin infections, and stomach disorders [1]. Previous investigations on this plant have revealed that this species was enriched with active monoterpene indole alkaloids (MIAs). So far, > 100 MIAs categorized in 17 types have been isolated from *R. stricta*. Some of *Rhazya* alkaloids such as aspidospermiose, leepacine, aspidospermidose, strictibine, strictanine, and strictanol are species-specific MIAs, which have been identified only from *R. stricta* [1–7]. The reported *Rhazya* alkaloids are capable of various therapeutic properties like antifungal, antimicrobial, antitumor, and antihypertensive properties [1]. During the course of our work for the discovery of structurally and biologically interesting alkaloids from medicinal plants [8,9], Twenty-seven MIAs (Fig. 1) including three new ones were isolated from *R. stricta*. Secopleiocarpamine A (**1**) is the first natural 2,3-*seco*-aspidosperma-type MIA possessing a cyano group. All MIAs isolated from this plant were screened for their inhibitory activities against six *Candida* strains. Herein, the isolation, structural elucidation, biological activities of **1**–**27**, and a plausible biogenetic pathway toward **1** are described.

2. Experimental

2.1. General experimental procedures

Optical rotations were measured on a Perkin-Elmer 341 polarimeter (PerkinElmer, Inc., Shelton, USA). UV spectra were recorded on a Shimadzu UV-2450 spectrophotometer. IR spectra were determined on a Bruker Tensor 37 infrared spectrophotometer. HRESIMS (70 eV) was recorded on a Finnigan MAT 95 mass spectrometer, and ESIMS was carried out on a Finnigan LCQ Deca instrument. 1D and 2D NMR spectra were measured on a Bruker AM-400 spectrometer at 25 °C. A Shimadzu LC-20 AT equipped with a SPD-M20A PDA detector was used for HPLC. An YMC-pack ODS-A column (250 × 10 mm, S-5 μm, 12 nm) was used for semi-preparative HPLC separation. Silica gel (300–400 mesh, Qingdao Haiyang Chemical Co., Ltd.), reversed-phase C_{18} (RP-C_{18}) silica gel (12 nm, S-50 μm, YMC Co., Ltd), Sephadex LH-20 gel (Amersham Biosciences), and MCI gel (CHP20P, 75–150 μm, Mitsubishi Chemical Industries Ltd.) were used for column chromatography (CC). All solvents used were of analytical grade (Guangzhou Chemical Reagents Company, Ltd. Guangzhou China).

2.2. Plant material

The whole plant of *R. stricta* was collected by Muhammad Iqbal and Muhammad Jamil in August 2016, from Pothohar Plateau of Punjab Province, Pakistan, and was identified by one of the author (G.-H. Tang). A voucher specimen (accession number: RS201601) has been deposited at the School of Pharmaceutical Sciences, Sun Yat-sen University.

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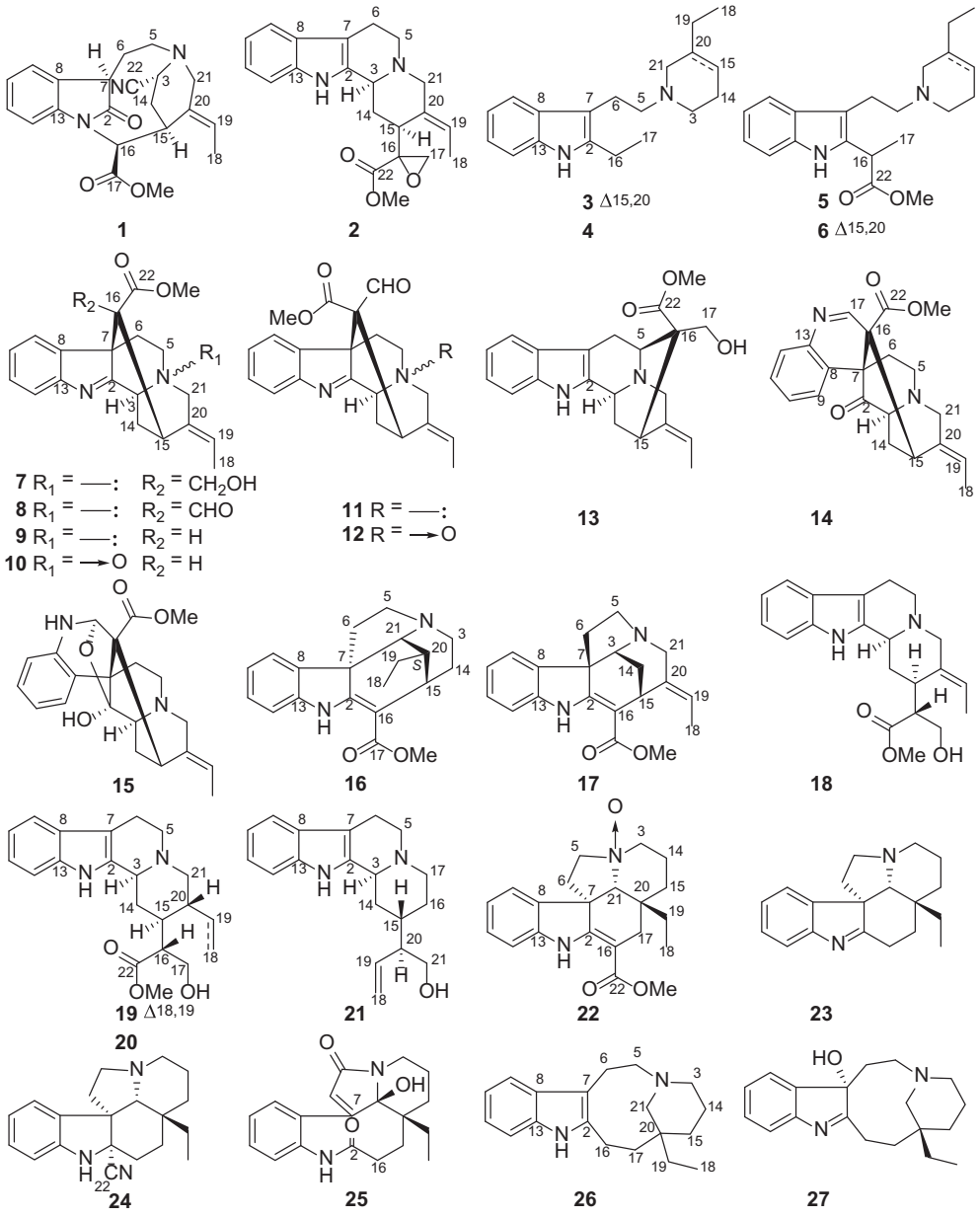

Fig. 1. Structures of compounds **1–27**.

2.3. Extraction and isolation

The dried grinded plant material (3 kg) was extracted exhaustively with 95% EtOH (10 L, three times). After filtration, the organic solvent was removed by using vacuum rotary evaporator to afford a total extract (300 g). The extract was further dissolved in water (1 L) and partitioned subsequently with petroleum ether (PE, 3 × 1.5 L) and EtOAc (3 × 1.5 L) to yield 7 g and 139 g of corresponding extracts, respectively. The EtOAc fraction was subjected to silica gel CC eluted with CH_2Cl_2/MeOH (1:100 → 100:0) to give two sub-fractions (Fr. AI–AII). Fr. AI (9 g) was subjected to Sephadex LH-20 (MeOH) and then chromatographed over silica gel CC (PE/ EtOAc, 10:1 → 0:100) to obtain **23** (100 mg) and **24** (11 mg) and a sub-fraction, which was further purified on HPLC using MeOH/H_2O (80:20, *v*/v; flow rate: 3 mL/min) as mobile phase to afford **1** (4 mg, t_R = 8 min), **5** (25 mg, t_R = 9 min), **6** (24 mg, t_R = 11 min), and **26** (10 mg, t_R = 13 min). Fr. AII (3 g) was subjected to Sephadex LH-20 (MeOH) to afford two sub-fractions (Fr. AII_1 and Fr. AII_2). Fr. AII_1 was undergone through silica gel CC (PE/EtOAc, 5:1 → 0:100) to obtain **3** (15 mg), **4** (58 mg), **16** (20 mg), and sub fraction, which was then purified using HPLC using MeOH/H_2O (85:15, *v*/v; flow rate: 3 mL/min) to get **13** (65 mg, t_R = 7 min), **9** (46 mg, t_R = 9 min), **19** (10 mg, t_R = 10 min) and **20** (10 mg, t_R = 12 min). Fr. AII_2 was loaded on to Sephadex LH-20 (MeOH) and chromatographed over HPLC using MeOH/H_2O (85:15, *v*/v; flow rate: 3 mL/min) to afford **8** (27 mg, t_R = 7 min), **11** (15 mg, t_R 9 = min), **17** (10 mg, t_R = 10 min) **12** (30 mg, t_R = 13 min), and **27** (15 mg, t_R = 15 min). The PE fraction was then loaded on to MCI column eluted with MeOH/H_2O (30:70 → 100:0) to afford four sub fractions (Fr. BI–BIV). Fr. BI (150 mg) was undergone through silica gel CC (CH_2Cl_2/MeOH, 250:1→ 0:100) to acquire **10** (18 mg) and **25** (7 mg). Fr. BII (200 mg) was subjected to Sephadax LH-20 (MeOH) to obtain **22** (22 mg) and **15** (80 mg). Fr. BIII (500 mg) was subjected to silica gel CC eluted with PE/EtOAc (20:1→0:100) and followed by HPLC using MeOH/H_2O (80:20, *v*/v; flow rate: 3 mL/min) to obtained compounds **7** (2 mg, t_R = 7mins) and **14** (7 mg, t_R = 9mins). Fr. BIV (1 g) was chromatographed over silica gel column (CH_2Cl_2/MeOH 200:1→0:100) and then subjected to