Phytochemistry xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Cytotoxic indole alkaloids from the fruits of *Melodinus cochinchinensis*

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ARTICLE INFO

Article history:
Received 26 September 2014
Received in revised form 18 February 2015
Available online xxxx

Keywords:
Melodinus cochinchinensis
Apocynaceae
Indole alkaloids
Cytotoxicity

ABSTRACT

Eight indole alkaloids, melosines A–H, together with 13 known alkaloids, were isolated from the fruits of *Melodinus cochinchinensis*. The structure elucidation of isolated secondary metabolites was based on comprehensive spectroscopic data analysis. Melosine B showed moderate cytotoxic activity against five human cancer cell lines, HL-60, SMMC-7721, A-549, MCF-7, and SW480 with IC_{50} values ranging from 1.6 to 8.1 μM.

1. Introduction

Melodinus cochinchinensis, belongs to the genus *Melodinus* (Apocynaceae), is mainly distributed in Indochina, W. Malaysia, E. Himalayas, and Yunnan of China, and the fruits are used as ethnic drugs for abdominal pain, infantile malnutrition due to intestinal parasites, indigestion, and hernia (Tsiang and Li, 1995). Previous chemical studies on the genus led to the isolation of novel and/or bioactive indole alkaloids (Liu et al., 2012, 2013). Until now, however, research on the chemical constituents in fruits of this plant has not been reported. As an extension of a search for bioactive indole alkaloids from the Apocynaceae (Fu et al., 2014; Guo et al., 2012a,b; Wang et al., 2011; Zhou et al., 2010), seven new compounds (**1**–**7**) (Fig. 1), including two new bisindole alkaloids, five new monomers, and a new natural product (**8**) (Fahn et al., 1990), together with 13 known alkaloids, namely 16β,21β-epoxy-vincadine (Cai et al., 2012), lochnerinine (Baassou et al., 1978; Moza et al., 1964), lochnericine (Kunesch et al., 1980), 16-epi-Δ^{14}-vincamine (Thissen et al., 2002; Li et al., 1989), (−)-vincapusine (Moldvai et al., 2006), Δ^{14}-vincamine (Bernauer et al., 1969), scandine (Achenbach et al., 1997), tabersonine-N-oxide (Wenkert et al., 1973), tabersonine (Wenkert et al., 1973), 3-oxo-tabersonine (Achenbach et al., 1997), 3-oxopachysiphine (Torrenegra et al., 1988), 3α-acetonyltabersonine (Fahn et al., 1990), and 11-methoxytabersonine (Kuehne et al., 1987; Pyuskyulev et al., 1967), were isolated from the fruits of *M. cochinchinensis*. Herein the isolation, structural elucidation, and the cytotoxicity evaluation were carried out in this present report.

2. Results and discussion

Compound **1** (melosine A) was isolated as a white powder. Its molecular formula was established as $C_{42}H_{42}N_4O_6$ by HREIMS (*m*/*z* 698.3116, $[M]^+$, calcd for $C_{42}H_{42}N_4O_6$ 698.3104), indicating 24 degrees of unsaturation. Its IR spectrum suggested the presence of amino (3446 cm^{-1}) and carbonyl (1670 cm^{-1}) functionalities. Its ^1H NMR spectrum indicated presence of two OCH_3 groups at δ_H 3.78 (6H, s) and two triplet methyls at δ_H 0.73 (H-18′, t, J = 7.2 Hz) and δ_H 0.82 (H-18, t, J = 7.2 Hz). The ^{13}C NMR and DEPT data displayed 42 carbon signals, which were classified as four methyls, six methylenes, 16 methines, and 16 quaternary carbons, respectively. Among them, 12 aryl carbons and six quaternary carbons (δ_C 90.3, 93.6, 160.1, 168.8, 169.0, and 169.9) were typical of two β-anilinoacrylate moieties (Fu et al., 2014). According to the analysis of its 2D NMR spectroscopic data (HSQC, HMBC, and ^1H–^1H COSY) as shown in Fig. 2, compound **1** was established as a bisindole alkaloid with two aspidospermine skeletons.

In unit A, two olefinic protons at δ_H 6.07 (1H, d, J = 10.0 Hz) and δ_H 6.83 (1H, d, J = 10.0 Hz) were assigned to the double bond at C-14/15 of the α,β-unsaturated ketone. The remaining olefinic proton at δ_H 7.34 (1H, s) was ascribed to one trisubstituted double bond connected to N-4 and C-7, which was confirmed by analysis of

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http://dx.doi.org/10.1016/j.phytochem.2015.02.028
0031-9422/

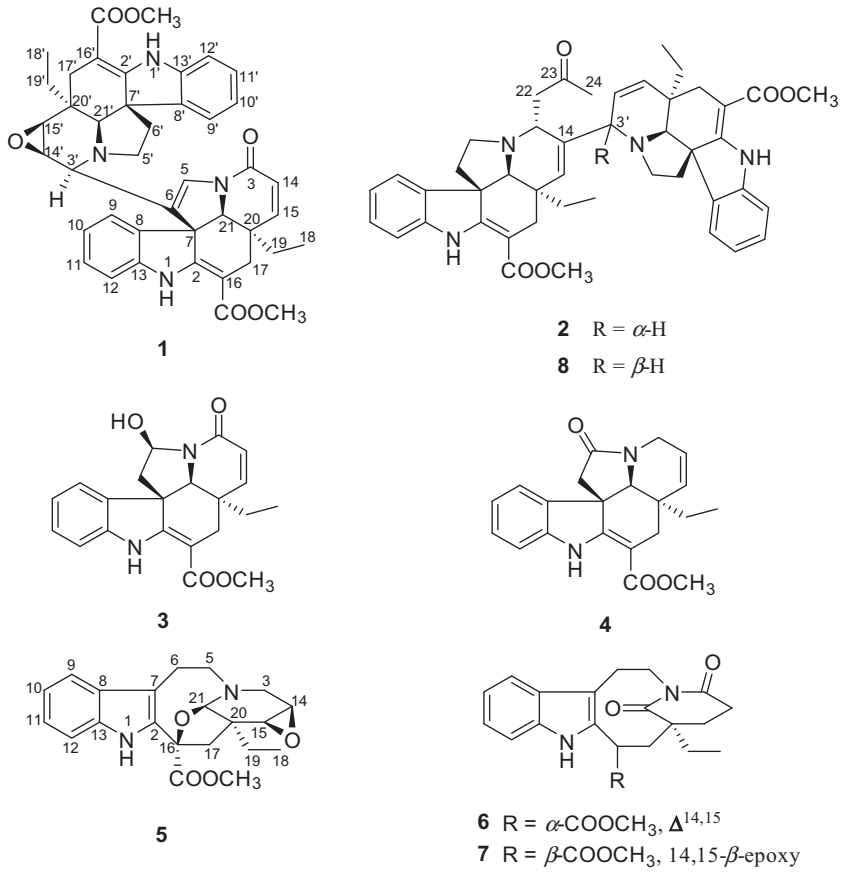

Fig. 1. Structures of compounds **1**–**8**.

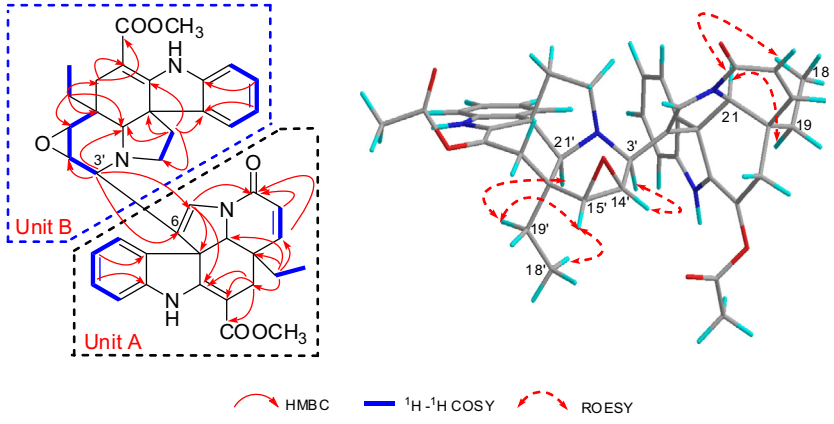

Fig. 2. Selected 2D NMR correlations for melosine A (**1**).

the HMBC correlations from H-5 (δ_H 7.34, s) to C-2 (δ_C 160.1), C-3 (δ_C 162.0), C-6 (δ_C 127.4), C-7 (δ_C 65.5), and C-21 (δ_C 67.6). According to these data, unit A was easy to determine as being 3-oxo-tabersonine (Achenbach et al., 1997) with a double bond between C-5 (δ_C 127.5) and C-6 (δ_C 127.4).

Two upshielded methines of C-14′ (δ_C 58.3) and C-15′ (δ_C 60.7) in unit B indicated the presence of an epoxy ring. In view of the remaining chemical shift patterns, unit B was characteristic of pachysiphine (Kunesch et al., 1980), this being confirmed by analysis of its 2D NMR spectra. Finally, a new linkage for bisindole alkaloids between C-6 in unit A and C-3′ in unit B was established by analysis of key HMBC correlations from H-3′ (δ_H 3.43, J = 5.3 Hz) to C-5 (δ_C 127.5) and C-6 (δ_C 127.4). The relative configuration of **1** was also determined by analysis of its ROESY spectrum as shown in Fig. 2. The α-orientation of H-21 and the ethyl group at C-20 was supported by the ROESY correlations of H-21/H-18 and H-21/H-19. Similarly, ROESY correlations of H-21′/H-19′b, H-15′/H-19′, H-15′/H-18′, and H-14′/H-3′ indicated that H-3′, H-14′, H-15′, and C-20′ ethyl group were α-oriented, while the epoxy moiety was β-oriented.

The molecular formula of melosine B (**2**), $C_{45}H_{50}N_4O_5$, was determined by positive HRESIMS (m/z 749.3673, $[M+Na]^+$, calcd for $C_{45}H_{50}N_4O_5Na$ 749.3679), with 23 degrees of unsaturation. The ^1H NMR spectroscopic data displayed two amino protons at

Please cite this article in press as: Shao, S., et al. Cytotoxic indole alkaloids from the fruits of *Melodinus cochinchinensis*. Phytochemistry (2015), http://dx.doi.org/10.1016/j.phytochem.2015.02.028