Phytochemistry xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Phytochemistry

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

# Cytotoxic indole alkaloids from *Tabernaemontana officinalis*

Bing-Jie Zhang [a,b], Xi-Feng Teng [c], Mei-Fen Bao [a], Xiu-Hong Zhong [a,b], Ling Ni [a,b], Xiang-Hai Cai [a,*]

[a] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, People's Republic of China
[b] University of Chinese Academy of Sciences, Beijing 100049, People's Republic of China
[c] School of Traditional Chinese Medicine, Guangdong Pharmaceutical University, Guangzhou 510006, People's Republic of China

ARTICLE INFO

*Article history:*
Received 23 July 2014
Received in revised form 12 November 2014
Available online xxxx

*Keywords:*
*Tabernaemontana officinalis*
Apocynaceae
Monoterpenoid indole alkaloid
Bisindole alkaloid
Cytotoxicity

ABSTRACT

Continued interest in cytotoxic alkaloids resulted in the isolation of 37 alkaloids, including 28 known monoterpenoid indole alkaloids from the aerial parts of *Tabernaemontana officinalis*. Of the remaining 9 alkaloids, six were bisindole alkaloids named taberdivarines A–F (**1**–**6**) and three were monomers named taberdivarines G–I (**7**–**9**). Alkaloids **1** and **2** are voaphylline–vobasinyl type bisindole alkaloids, a structural type previously unknown, while **3**–**6** exhibited cytotoxicity against three human cancer cell lines HeLa, MCF-7, and SW480 with $IC_{50}$ values ranging from 1.42 to 11.35 μM.



## 1. Introduction

Monoterpenoid indole alkaloids (MIAs) are of great significance in natural medicine and are well known for their distinctive structural features and significant diverse bioactivities (Saxton, 1995). Bisindole alkaloids, such as the vincristine and vinblastine derivatives, have attracted substantial attention due to their antitumor activities (Tanaka et al., 2009; Jordan and Kamath, 2007). A series of cytotoxic MIAs and their dimeric analogues were previously reported (Bao et al., 2013). Various bisindole alkaloids which exhibited diverse and fascinating molecular architecture have been isolated, such as akuammidine–ibogan, euburnane–ibogan, aspidospermatan–aspidospermatan and euburnane–aspidospermatan, vobasine–strychnan type alkaloids. More interestingly, some bisindole alkaloids rather than those with monomeric units showed cytotoxicity (Condello et al., 2014; Ma et al., 2014), which suggest that dimer formation might be key for bioactivity. Therefore, novel active dimers consisting of known and/or new monomers in various combinations deserve attention. Plants of the genus *Tabernaemontana* are rich in MIAs, particularly dimeric ones (VanBeek et al., 1984; Kam et al., 2003a). As part of a continuing search for new cytotoxic alkaloids, six new bisindole alkaloids and three new monomeric indole alkaloids, as well as 28 known alkaloids, were isolated from the leaves and twigs of *Tabernaemontana officinalis*. The known alkaloids were identified as voaphylline (**10**) (Kunesch et al., 1967a), 2α,7α-dihydroxydihydrovoaphylline (**11**) (Cai et al., 2012), voaphylline hydroxyindolenine (**12**) (Kunesch et al., 1967b), apparicine (**13**) (Joule et al., 1965), 3-(2-oxopropyl)coronaridinehydroxyindolenine (**14**) (Huang et al., 2006), vobasine (**15**) (Ahond et al., 1976), tabernaemontanine (**16**) (Ahond et al., 1976), dregamine (**17**) (Ahond et al., 1976), coronaridine (**18**) (Sharma and Cordell, 1988), heyneanine (**19**) (Gunasekera et al., 1980), isovocangine (**20**) (Ladhar et al., 1981), isovoaristine (**21**) (Wenkert et al., 1979), voacristine (**22**) (Wenkert et al., 1979), 3-oxo-coronaridine (**23**) (Sharma and Cordell, 1988), coronaridinehydroxyindolenine (**24**) (Sharma and Cordell, 1988), divaricatin F (**25**) (Bao et al., 2013), ibogamine (**26**) (Liang et al., 2007), 20-epi-ervatarnine (**27**) (Knox and Slobbe, 1975), 19-dehydroervatamine (**28**) (Knox and Slobbe, 1975), ervatarnine (**29**) (Knox and Slobbe, 1975), 3-(2-oxopropyl)coronaridine (**30**) (Ahond et al., 1976), 19-acetonylvoacangine (**31**) (Okuyama et al., 1992), ervataminic acid (**32**) (Knox and Slobbe, 1975), conofoline (**33**) (Kam et al., 2003b), 19,20-dihydroervahanine A (**34**) (Henriques et al., 1996), tabernaelegantine D (**35**) (Bombardelli et al., 1976), ervadivaricatine A (**36**) (Huang et al., 1997), and ervadivaricatine B (**37**) (Huang et al., 1997), respectively. The cytotoxicity of these alkaloids against five human cancer cell lines was evaluated in this study.

## 2. Results and discussion

The alkaloid fraction of *T. officinalis* was separated as described in Section 3 to yield a total of 37 compounds, including nine new alkaloids **1**–**9** (Fig. 1). All compounds were most likely alkaloids as they exhibited a positive reaction with Dragendorff's reagent.

* Corresponding author. Tel.: +86 871 65223188; fax: +86 871 65150227.
*E-mail address:* xhcai@mail.kib.ac.cn (X.-H. Cai).

http://dx.doi.org/10.1016/j.phytochem.2014.12.025
0031-9422/

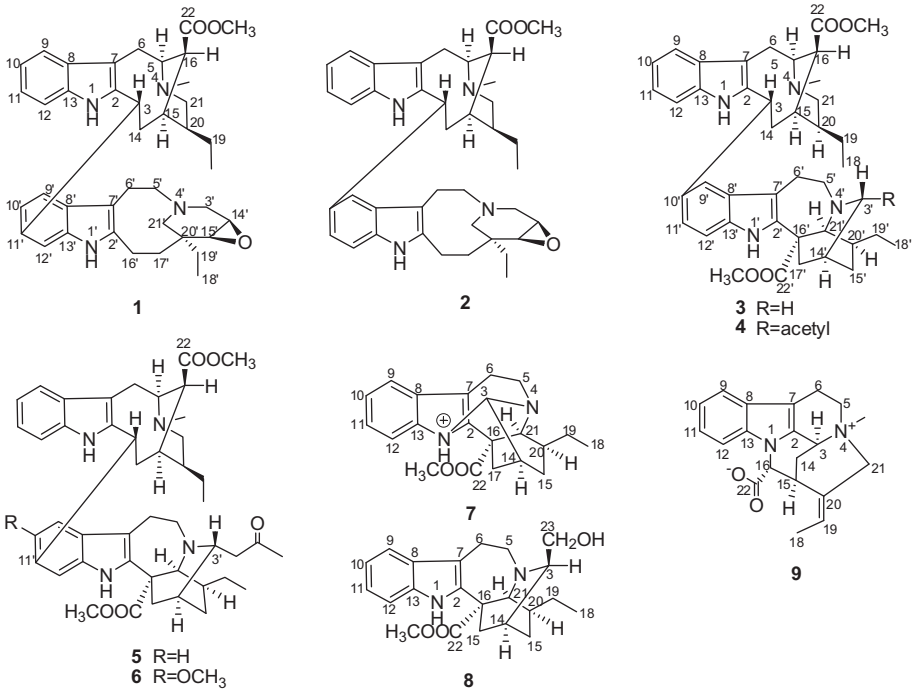


**Fig. 1.** Alkaloids (**1**–**9**) isolated from *T. officinalis*.

The UV absorption bands at 289, 227, and 208 nm and the IR vibrations at 3397 and 1721 $cm^{-1}$ of **1** were consistent with the presence of an indole moiety (Albinsson and Norden, 1992). The positive HRESIMS ($[M+H]^+$ at $m/z$ 635.3969) and $^{13}C$ NMR spectroscopic data established its molecular formula as $C_{40}H_{50}N_4O_3$. Its $^1H$ NMR data (Table 1) indicated the presence of a non-substituted indole ring A [$\delta_H$ 7.46 (d, $J$ = 7.1 Hz), 6.91 (t, $J$ = 7.1 Hz), 6.93 (t, $J$ = 7.1 Hz), and 7.03 (d, $J$ = 7.1 Hz)], a mono-substituted ring A′ [$\delta_H$ 7.19 (d, $J$ = 8.1 Hz), 6.73 (d, $J$ = 8.1 Hz), and 6.88 (s)], two indolic NH protons [$\delta_H$ 10.48 (s) and 10.17(s)], a nitrogen menthyl ($\delta_H$ 2.44, s), and a methyl ester group ($\delta_H$ 2.34, s), respectively (Fig. 2). These data indicated that **1** was an MIA dimer. In addition, further analysis of the DEPT and $^{13}C$ NMR spectroscopic data (Table 2) of **1** established the presence of ten quaternary carbons, a methyl ester group, fourteen methines, eleven methylenes, four methyls indicating the presence of vobasine and voaphylline units. The only upfield quaternary carbon, at $\delta_C$ 33.2, is a characteristic resonance of C-20′ in the voaphylline unit (Kunesch et al., 1967a), and the presence of seven methines $\delta_C$ from ~60 to 30 also indicated that the other unit was a vobasine (sarpapan)-type (Sroll and Hofmann, 1953). The vobasine and voaphylline units were further confirmed by analysis of its HMQC, HMBC, and ROESY spectra and assignment of the $^1H$ and $^{13}C$ NMR spectroscopic data. The connectivity of both units was established during NMR assignment and was further supported by the HMBC spectroscopic data (S3 and S4, Supporting information), where correlations from $\delta_H$ 4.41 (H-3) to $\delta_C$ 118.1 (C-10′) and $\delta_C$ 109.0 (C-12′) and from $\delta_H$ 6.88 (s, H-12′) to C-10′ and $\delta_C$ 126.4 (C-8′) verified the C-3/11′ connectivity. The relative configuration of **1** was determined through analysis of the $^1H$ and $^{13}C$ NMR spectroscopic data and the ROSEY spectra. H-3 was *trans*-diaxial relative to 14α-H based on the large coupling constant ($J$ = 13.0 Hz), which indicated that H-3 was in the β-orientation (Kam and Sim, 2003). H-20 was determined to be in α-orientation by examination of the resonances at C-14 ($\delta_C$ 42.1) and C-16 ($\delta_C$ 43.2), which were closer to those of tabernaemontanine than to dregamine based on comparison of the C-14/16 chemical shifts of the two isomers (Ahond et al., 1976). The ROSEY correlations from H-16 to H-5 and H-19 established the stereochemistry of **1** to be that as shown in Fig. 2. Furthermore, the shielded proton resonance ($\delta_H$ 2.34) of the methyl ester group suggested that it was in a β-orientation (Nugroho et al., 2009). In addition, in the voaphylline unit, correlations of H-14′/H-15′ and H-15′/H-19′ placed both H-15′ and H-14′ in an α-orientation. The above data established the structure of **1** to be that shown or its enantiomer, and **1** was named taberdivarine A.

Alkaloid **2** had the same molecular formula as **1**, $C_{40}H_{50}N_4O_3$, based on $^{13}C$ NMR spectroscopic data the an ion peak at $m/z$ 635.3972 $[M+H]^+$, as established by HRESIMS. Similar to **1**, the

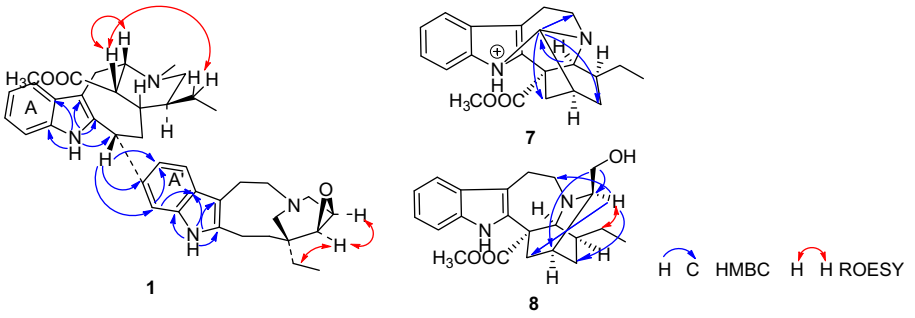


**Fig. 2.** Key HMBC and ROESY correlations of **1**, **7** and **8**.