Cytotoxic monoterpenoid indole alkaloids isolated from the barks of *Voacanga africana* Staph.

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A new monoterpenoid indole alkaloid compound (1) and six known monoterpenoid indole alkaloids compounds (2–7) were isolated from the barks of *Voacanga africana* Staph. The structures were established by spectral analysis as ibogamine-16-carboxylic acid,17,20-didehydro-5,6-dioxo-10-methoxy-methyl ester (1), voacamine (2), vobasine (3), voacangine (4), voacristine (5), 19-epi-voacristine (6) and 19-epi-heyneanine (7). Compound 1 was confirmed by X-ray crystallographic analysis. All of the isolated compounds were evaluated for cytotoxicity against five cell lines (HEPG-2, A375, MDA-MB-231, SH-SY5Y, CT26). Among them, compounds 2 and 6 displayed significant inhibitory activities, compounds 3, 4 and 5 showed moderate inhibitory activities, while compounds 1 and 7 showed no inhibitory activities against the five cell lines.

**Keywords:** *Voacanga africana* Staph; monoterpenoid indole alkaloids; cytotoxicity

1. Introduction

*Voacanga africana* Staph. from the family Apocynaceae is widespread in West Africa, Congo and Tanzania. The plant is widely distributed over secondary forests and transitional zones (Adolfina et al. 2009). It has been traditionally used for the treatment of leprosy, diarrhoea, generalised oedema, convulsions in children, madness, diuretic and infant tonic (Iwu 1993; Neuwinger 2000; Tan et al. 2000). *V. africana* Staph. is a very rich source of a number of indole alkaloids with intriguing carbon skeletons and biological activities (Kang et al. 2012; Mei et al. 2012). In our previous investigation on this plant, 11 alkaloids with cancer toxicity were obtained (Mei 2012; Dan 2014). Now, further investigation on the ethyl acetate extract of

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V. africana Staph. led to the isolation of a new monoterpenoid indole alkaloid 1 and six known monoterpenoid indole alkaloid compounds 2–7 (Figure 1). Furthermore, these seven compounds were evaluated in vitro against the HEPG-2, A375, MDA-MB-231, SH-SY5Y and CT26 tumour cell lines. We herein report the isolation, structural elucidation and bioactivity of these compounds.

2. Results and discussion

Compound 1 was obtained as colourless crystals. mp 341–342°C. The molecular formula was determined as C_{22}H_{24}N_{2}O_{5} on the basis of HR-ESI-MS (m/z 395.1620 [M – H]–, calcd for 395.1601; m/z 419.1554 [M + Na]+, calcd for 419.1577). Its UV spectrum showed the maximum absorption bands at 211 and 262 nm. The IR spectrum of compound 1 showed absorptions of NH group (3191 cm–1), carbonyl groups (1749, 1625 cm–1) and aromatic ring (1588, 1455 cm–1). In the 1H NMR spectrum of 1, three aromatic proton signals at δ 7.39 (1H, d, J = 2.3 Hz, H-9), δ 6.85 (1H, dd, J = 8.8, 2.3 Hz, H-11) and δ 7.29 (1H, d, J = 8.8 Hz, H-12) were observed. In addition, two methoxy at δ 3.63 (3H, s) and δ 3.77 (3H, s), a bridgehead proton adjacent to nitrogen at δ 4.20 (1H, brs, H-21) and ethyl signals at δ 0.88 (3H, t, J = 7.3 Hz) and δ 1.52 and δ 1.39 (each 1H, m, CH_{2}) were demonstrated. The 13C NMR spectrum of 1 revealed eight unsaturated carbon signals (δ_{C} 101.9, 110.6, 113.7, 113.7, 126.6, 131.1, 151.7, 156.4), suggesting that 1 was an indole alkaloid coupling with the 1H NMR spectroscopic data of 1. The 13C NMR spectroscopic data of 1 was similar to corresponding data of ibogamine-18-carboxylic acid,3,4-didehydro-7,8-dioxo-methyl ester (Huang et al. 2006) except for C-10, while the significant difference was the presence of signal at δ156.39 in 1 instead of the signal at δ126.0 in ibogamine-18-carboxylic acid,3,4-didehydro-7,8-dioxo-methyl ester. In the HMBC correlations (Figure S10), the correlation between the proton of –OCH_{3} (δ_{H} 3.77, 3H, s) and C-10 (δ_{C} 156.39) confirmed that the methoxy group was attached to C-10. The HMBC spectrum also showed correlations of indole ring arising from H-9 (δ_{H} 7.39) to C-7(δ_{C} 110.56), C-10(δ_{C} 156.39), C-11(δ_{C} 113.71) and C-13(δ_{C} 131.07), H-11(δ_{H} 6.85) to C-9(δ_{C} 101.86), C-10

Figure 1. Chemical structures of compounds 1–7.
(δ_C 156.39) and C-13(δ_C 131.07), H-12(δ_H 7.29) to C-8(δ_C 126.57), C-9(δ_C 101.86) and C-10 (δ_C 156.39). On the basis of the results, the structure of compound 1 was identified as ibogamine-16-carboxylic acid,17,20-didehydro-5,6-dioxo-10-methoxy-methyl ester. The structure was ultimately confirmed by single crystal X-ray diffraction study of 1 where the α-orientation of C-16 methyl ester and H-21 along with the β-orientation of C-3 and C-19 methylene were clearly visible (Figure 2).

Six known compounds (2–7) were identified as voacamine (2) (Medeiros et al. 1999), vobasine (3) (Liang et al. 2007), voacangine (4) (Liang et al. 2007), voacristine (5) (Janot & Goutarel 1995), 19-epi-voacristine (6) (Perera et al. 1983) and 19-epi-heyneanine (7) (Liang et al. 2007), in comparison with their physical constant data in the literature.

The cytotoxicity of compounds 1–7 was evaluated against HEPG-2, A375, MDA-MB-231, SH-SY5Y and CT26 cancer cell lines using the MTT method. The results are listed in Table 1. Compound 2 possessed significant cytotoxic activity against HEPG-2, A375, MDA-MB-231, SH-SY5Y and CT26. Compound 3 showed moderate cytotoxic activity against HEPG-2, A375, MDA-MB-231, SH-SY5Y and CT26. Compounds 1, 4–7 share the same basic skeleton with different substitution patterns, yet their cytotoxic activities varied greatly. Compound 6 exhibited significant activity against HEPG-2, A375, MDA-MB-231, SH-SY5Y and CT26. Compound 4 exhibited moderate activity against A375 and CT26 and significant activity against HEPG-2, MDA-MB-231 and SH-SY5Y. Compound 5 exhibited moderate activity against HEPG-2, A375, MDA-MB-231, SH-SY5Y and CT26. None of the tested cell lines were susceptible to compounds 1 and 7.

Table 1. Cytotoxicity of compounds 1–7.

| Compound | HEPG-2 | A375 | MDA-MB-231 | SH-SY5Y | CT26 |
|----------|--------|------|------------|---------|------|
| 1        | >100   | >100 | >100       | >100    | >100 |
| 2        | 5.7 ± 0.9 | 5.0 ± 1.7 | 5.5 ± 1.4 | 5.2 ± 1.9 | 5.5 ± 1.1 |
| 3        | 19.3 ± 2.5 | 19.5 ± 4.6 | 25 ± 7.2 | 19 ± 4.9 | 20 ± 1.3 |
| 4        | 10 ± 0.8  | 14 ± 4.3  | 8.0 ± 1.1 | 7.6 ± 2.6 | 11 ± 3.4 |
| 5        | 20 ± 2.1  | 20 ± 1.3  | 20 ± 2.5  | 20 ± 5.7  | 20 ± 4.4 |
| 6        | 10.5 ± 1.6 | 5.0 ± 1.9 | 6.3 ± 1.2 | 3.8 ± 0.7 | 4.8 ± 1.5 |
| 7        | >100     | >100     | >100       | >100     | >100 |
| Taxol    | 0.17 ± 0.05 | 0.83 ± 0.16 | 0.62 ± 0.11 | 0.21 ± 0.05 | 0.57 ± 0.10 |

Note: Data are given as mean ± SD.
3. Experimental

3.1. General experimental procedure

Column chromatography was performed on silica gel (200–300 mesh, Qingdao Marine Chemical Group Co., Qingdao, China), Sephadex LH-20 (GE Healthcare UK Ltd, Buckinghamshire, UK) and MCI GEL CHP20P (75–150 μm, Mitsubishi Chemical Holdings, Tokyo, Japan). HPLC separation was performed in a Model LC-10ATVP system coupled with a UV–vis detector. A YMC-Pack ODS-A semi-preparative HPLC column (250 mm × 10 mm, S-5 μm, 12 nm) was used. UV spectra were recorded on a UV1100 spectrometer (Shanghai Tianmei Scientific Instrument Inc., Shanghai, China). IR spectra were measured on an FT-IR spectrometer (PerkinElmer, Waltham, MA, USA) using KBr pellets. NMR spectra were obtained on a Bruker 400/600 MHz spectrometer (Bruker Biospin, Faellanden, Switzerland) with tetramethylsilane as internal standard. HR-ESI-MS was recorded on a micrOTOF-Q II10339 premier mass spectrometer (Bruker Daltonics, Bremen, Germany). Optical rotations were recorded on a Perkin Elmer 341 polarimeter (PerkinElmer). X-ray crystallographic analysis was determined by Bruker Smart APEX II CCD (Bruker Biospin, Faellanden, Switzerland).

3.2. Plant material

The barks of *V. africana* Staph. were purchased from Ghana, Africa in April 2012 and were identified by Dr Hai-Feng Liu, Sichuan University. A voucher specimen (no. FKS-20120421-JN) has been deposited in the Laboratory of Chinese Medicinal Chemistry, Pharmaceutical College, Chengdu University of Traditional Chinese Medicine.

3.3. Extraction and isolation

The dried and powdered barks of *V. africana* Staph. (14.5 Kg) were percolated with 10 times amount of EtOAc, the extract was evaporated to afford 546 g of crude extract. The crude was subjected to silica gel CC eluted with petroleum ether/EtOAc in linear gradient (5:1, 4:1, 3:1, 2:1, 1:1, 0:1) to obtain eight fractions (Fr.1–8) on the basis of TLC profiles. Fraction 1 dissolved with acid (HCl, 0.3 mol/L) and precipitated with NaOH and recrystallised from methanol to yield compound 4 (11 g). Fraction 2 was chromatographed on silica gel column and eluted with petroleum ether/acetone (6:1, 5:1, 4:1, 3:1, 2:1, 1:1) to furnish six sub-fractions, designated as (Fr.2-1 to Fr.2-6), compound 5 (12 g) was crystallised from Fr.2-6. Fraction 3 was applied to Sephadex LH-20 (CHCl 3/MeOH, 1:1) to get compounds 6 (220 mg) and 7 (200 mg). Fraction 4 was subjected to silica gel column and eluted with petroleum ether/EtOAc (10:1, 8:1, 6:1, 4:1, 2:1, 1:1) to yield three sub-fractions (Fr.4-1 to Fr.4-3), Fr.4-1 was subjected to RP-8 column using MeOH/H 2O (40:60, 50:50, 60:40, 70:30, 80:20, 90:10, 100:0) as eluent to afford compound 1 (18.8 mg). Fr.4-2 was applied to Sephadex LH-20 (MeOH) to get compound 2 (200 mg). Fr.4-3 was further purified by semi-preparative HPLC to afford compound 3 (20 mg).

3.3.1. *Ibogamine-16-carboxylic acid,17,20-didehydro-5,6-dioxo-10-methoxy-methyl ester (I)*

Colourless crystals, [α]D20 + 131.36° (c = 1.1, MeOH), mp 341–342°C. UV (MeOH) λmax nm: 211, 262; IR (KBr, Vmax, cm−1): 3191, 1749, 1625, 1588, 1455; HR-ESI-MS: m/z 395.1620 [M − H]−, (calcd for C22H23N2O5, 395.1601); m/z 419.1554 [M + Na]+, (calcd for C23H24N2O5Na, 419.1577). 1H NMR (600 MHz, DMSO-d6): 7.39 (1H, d, J = 2.3 Hz, H-9), 7.29 (1H, d, J = 8.8 Hz, H-12), 6.85 (1H, dd, J = 8.8, 2.4 Hz, H-11), 4.20 (1H, brs, H-21), 3.77 (3H, s, 10-OCH 3), 3.63 (3H, s, −COOCH3), 3.50 (1H, d, J = 11.4 Hz, H-3b), 3.14 (1H, d, J = 11.6 Hz, H-3a), 2.96 (1H, m, H-15b), 2.29 (1H, s, H-14), 1.82 (1H, m, H-17a), 1.81 (1H, m, H-15a), 1.67 (1H, m, H-20), 1.52 (1H, m, H-19a), 1.39 (1H, m, H-19b), 1.37 (1H, m, H-17b),
0.88 (3H, t, H-18). 13C NMR (150 MHz, DMSO-d6): 185.1 (C-6), 172.0 (−COOCH3), 168.4 (C-5), 156.4 (C-10), 151.7 (C-2), 131.1 (C-13), 126.6 (C-8), 113.7 (C-12), 113.7 (C-11), 110.6 (C-7), 101.9 (C-9), 56.1 (C-21), 55.7 (-10-OCH3), 53.5 (−COOCH3), 52.1 (C-16), 48.1 (C-3), 35.5 (C-20), 33.1 (C-15), 28.8 (C-17), 28.1 (C-14), 28.0 (C-19), 12.0 (C-18).

3.4. Crystal data for 1
X-ray data were collected at room temperature with Mo Kα radiation (graphite monochromator λ = 0.71073 Å) on a Huber four circle diffractometer equipped with a Bruker APEX-II CCD area detector. Data reduction was carried out using Bruker SAIN.

Structural solution and refinement (programs SHELXS-97 and SHELXL-2013) ran routinely.

C22H24N2O5, Mr = 396.43, colourless crystals were grown from EtOAc. Space group ‘orthorhombic’ ‘P21’. Lattice constants (Å): a = 7.2550 (9), b = 14.7177 (18), c = 18.663 (2), α = 90, β = 90, γ = 90, cell volume V = 1992.78 (40) Å³, formula units/cell Z = 4, 16,880 reflections collected, 4983 reflections unique (Rint = 0.0654), after convergence R1 = 0.0483 (3285), wR2 = 0.1173 (4983).

3.5. MTT assay
The cancer cell lines HEPG-2, A375, MDA-MB-231, SH-SY5Y and CT26 were obtained from the American Type Culture Collection, USA. Cultures were incubated in a humidified atmosphere of 5% CO2 at 37°C. When the cells enter the logarithmic growth phase, cells (3 × 10³/well) were seeded in supplemented culture medium (100 μL/well) in a 96-well plate and incubated for cells adherent. The medium was then replaced with a test compound-containing medium, and the cells were further incubated for 72 h. DMSO 10% was used as blank sample while Taxol was used as positive control. The cell viabilities were evaluated by MTT assays. The plate was then read on a microplate reader at 570 nm to evaluate the effects of the test compounds on cell growth. Experiments were conducted in triplicate.

4. Conclusions
A new compound and six known compounds were isolated from the barks of V. africana Staph. They were bis monoterpenes indole alkaloid (compound 2) and monoterpenes indole alkaloid (compounds 1, 3–7). All the compounds were tested for their in vitro cytotoxic activities against the HEPG-2, A375, MDA-MB-231, SH-SY5Y and CT26 cancer cell lines. Ibogamine monoterpenoid indole alkaloids showed important cytotoxicities against tumour cells, the presence methyl ester of C-16 was necessary to maintain the activities (Wang 2008). Compound 2 was bis monoterpenes indole alkaloid, which showed stronger activity than monoterpenes indole alkaloids.

Supplementary material
The UV, IR, HR-ESI-MS, 1H NMR, 13C NMR, 13C DEPT 90°, 13C DEPT 135°, HSQC and HMBC spectra of 1 can be found in the online-only supplementary material, Figures S1–S10. Crystallographic data in CIF format are available free of charge via the Internet at CCDC 1016804. These data can be obtained free of charge via https://www.ccdc.cam.ac.uk/services/structure_deposit/ (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033; or deposit_reply@ccdc.cam.ac.uk).
Disclosure statement
No potential conflict of interest was reported by the authors.

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