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Three new monoterpenoid indole alkaloids from *Vinca major*

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1. Introduction

Plants of the family Apocynaceae are rich sources of structurally diversified indole alkaloids, which originate from the condensation of tryptophan with secologanin \cite{1}. Many of them, such as yohimbine \cite{2}, reserpine \cite{3}, and camptothecin, \cite{4} are well known for their pharmacological significance. *Vinca major* is a perennial evergreen herb with medicinal and ornamental values and has also been cultivated in China widely as an outside ornament. Previous phytochemical investigations on this species collected from Turkey \cite{5}, Yugoslavia \cite{6}, Canada \cite{7}, Iran \cite{8}, India \cite{9}, and Japan \cite{10} reported diverse alkaloidal patterns, such as yohimbine, sarpagine, ajmaline, akuammine, and oxindole types. In our previous study, we have reported the isolation of two new monoterpenoid indole alkaloids, vincamajorines A and B, which represented two new carbon skeletons \cite{11}. As a continuation of our search for more effective components from this plant, three new monoterpenoid indole alkaloids, 19-hydroxyl-10-methoxy-19,20-dihydrovinorine (1), 19-O-acetyl-10-methoxy-19,20-dihydrovinorine (2), and 19, 21\(\alpha\)-dihydroxy-10-methoxy-19,20-dihydrovinorine (3), along with five known analogues (4–8), were isolated from the whole plants of *Vinca major*. The new structures were elucidated by extensive NMR and MS analysis and comparison with known compounds. In addition, compounds 1–3 were evaluated for their cytotoxicities against five human cancer cell lines.

**ABSTRACT**

Three new monoterpenoid indole alkaloids, 19-hydroxyl-10-methoxy-19,20-dihydrovinorine (1), 19-O-acetyl-10-methoxy-19,20-dihydrovinorine (2), and 19, 21\(\alpha\)-dihydroxy-10-methoxy-19,20-dihydrovinorine (3), along with five known analogues (4–8), were isolated from the whole plants of *Vinca major*. The new structures were elucidated by extensive NMR and MS analysis and comparison with known compounds. In addition, compounds 1–3 were evaluated for their cytotoxicities against five human cancer cell lines.

**KEYWORDS**

Apocynaceae; *Vinca major*; monoterpenoid indole alkaloid; cytotoxicity

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five human cancer cell lines. Reported herein are the isolation, structural elucidation, and cytotoxic activities of three new compounds.

2. Results and discussion

Compound 1 was obtained as colorless oil. The molecular formula C$_{22}$H$_{26}$N$_{2}$O$_{4}$ was determined by the molecular ion peak at $m/z$ 382.1894 [M]$^+$ in the HREIMS, in combination with $^1$H and $^{13}$C NMR spectra (Table 1), indicating 11 degrees of unsaturation. The IR spectrum displayed absorption bands at 3440 (–OH) and 1678 (C=O) cm$^{-1}$, while the UV absorption maxima at $\lambda$ 279, 222, and 204 nm indicated the presence of a substituted indolenine chromophore [12]. The $^1$H and $^{13}$C NMR (including DEPT spectrum) spectral data of compound 1 further confirmed the existence of the substituted indolenine ring [δ$_C$ 176.2 (s, C-2), 65.2 (s, C-7), 137.9 (s, C-8), 111.8 (d, C-9), 160.7 (s, C-10), 115.3 (d, C-11), 122.9 (s, C-12) and 149.8 (s, C-13); δ$_H$ 7.15 (d, $J$ = 2.4 Hz, H-9), 7.00 (dd, $J$ = 8.5, 2.4 Hz, H-11), and 7.53 (d, $J$ = 8.5 Hz, H-12)]. Besides the signals of the indolenine ring, the $^{13}$C NMR spectrum displayed 14 additional carbons, which were classified by the chemical shifts and HSQC spectrum as seven methines (δ$_C$ 77.7, 58.9, 57.8, 56.1, 49.8, 44.7, and 27.4), three methylenes (δ$_C$ 60.9, 36.2, and 21.9), one methyl (δ$_C$ 16.0), one O-methyl group (δ$_C$ 56.2), and one O-acetyl group (δ$_C$ 171.3, 20.7). The $^1$H-$^1$H COSY correlations established the linkages of H-3/H-14/H-15/H-16/H-5/H-6 and H-18/H-19/H-20/H-21 fragments, as shown in Figure 2. The 1D and 2D NMR data are suggestive that 1 possesses the same carbon skeleton with 10-methoxyvinorine (4) [12]. The main difference between the two compounds reflected in the chemical shifts of C-19 (δ$_C$ 116.4 (d) in 4; 57.8 (d) in 1) and C-20 (δ$_C$ 137.8 (s) in 4 and 44.7 (d) in 1), which suggested that the double bond between C-19 and C-20 in 4 has been saturated in 1. Meanwhile, C-19 of 1 was substituted by a hydroxyl group, in accordance with the molecular formula and the oxygenated nature of C-19. Accordingly, the chemical shift values of C-18 (δ$_C$ 116.4) and C-21 (δ$_C$ 60.9) in 1 shifted downfield compared to 4 (δ$_C$ 13.0 (q, C-18) and 54.0 (d, C-21)) based on the above reason.

The HMBC cross-peaks (Figure 2) of Me-18 (δ$_H$ 1.57, d, $J$ = 6.5 Hz) with C-19 and C-20, and of H-15 (δ$_H$ 2.61–2.67, m) and H$_b$-21 (δ$_H$ 3.73–3.78, m) with C-20, further confirmed the above conclusion. Additionally, the O-methyl group and O-acetyl group were located at C-10 and C-17 (δ$_C$ 77.7, d), respectively, according to the HMBC correlations from the methoxyl (δ$_H$ 3.83, s) to C-10, as well as from H-17 (δ$_H$ 5.11, d, $J$ = 1.3 Hz) and the acetyl methyl (δ$_H$ 2.20, s) to the carbonyl carbon at δ$_C$ 171.3.

![Figure 1. Structures of compounds 1–8.](image)
The relative configuration of 1 was established by ROESY experiment (Figure 3) and comparing with compound 4. The NOE correlation of H-3/H-20 indicated the α-orientation of H-20. However, the relative configuration of C-19 could not be determined, and attempts to grow a single crystal have so far not been successful. Thus, the structure of 1 was determined to be 19-hydroxyl-10-methoxy-19, 20-dihydrovinorine.

The molecular formula of compound 2 was determined as C_{24}H_{28}N_2O_5 by the molecular ion peak at m/z 424.1989 [M]+ in the HREIMS, requiring 12 degrees of unsaturation. The 1H and 13C NMR spectra of 2 (Table 1) showed similar patterns to those of 1, and the only difference was the presence of the other O-acetyl group ([δ_H 2.06 (s); δ_C 173.0 (s) and 20.9 (q)]) in 2. The connection between the O-acetyl and C-19 (δ_C 54.3, d) was established by the
HMBC correlations from the protons at $\delta_H$ 2.67–2.72 (m, H-19) and 2.06 (s, -OCOMe) to the carbonyl group at $\delta_C$ 173.0 (s). The ROESY data showed that the relative configuration of 2 was the same as compound 1. Therefore, the structure of 2 was elucidated as 19-O-acetyl-10-methoxy-19, 20-dihydrovinorine.

The HR-EI-MS of 3 gave a molecular ion peak at m/z 398.1834, corresponding to a molecular formula of C$_{22}$H$_{26}$N$_2$O$_5$. A side-by-side comparison of the $^1$H and $^{13}$C NMR spectra of 3 (Table 1) with those of 1 showed extremely structural similarity. The absence of the methylene at C-21 ($\delta_C$ 60.9, t) and the appearance of an oxygenated methine at $\delta_C$ 100.1, suggested that C-21 of compound 3 was substituted by a hydroxyl group, which was further supported by the HMBC correlations from H-15 ($\delta_H$ 2.48–2.52, m), H-19 ($\delta_H$ 2.86–2.91, m), and H-20 ($\delta_H$ 1.40–1.46, m) to C-21. The NOE correlations between H-3/H-20 and H-5/H-21 suggested the $\alpha$-position for H-20 and $\beta$-orientation for H-21, respectively. The structure of compound 3 was thereby concluded to be 19, 21-21$\alpha$-dihydroxyl-10-methoxy-19, 20-dihydrovinorine.

Compounds 1–3 were assayed for their cytotoxicities against five human tumor cell lines (MCF-7, SMMC-7721, HL-60, SW480, and A-549) by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) method. Unfortunately, all compounds were found to be inactive with IC$_{50}$ values greater than 40 μM.

3. Experimental

3.1. General experimental procedures

Optical rotations were obtained on a JASCO P-1020 digital polarimeter (JASCO, Tokyo, Japan). UV spectra were measured using a Shimadzu UV-2401A spectrophotometer (Shimadzu, Tokyo, Japan). IR spectra were recorded on a Bruker Tensor 27 spectrophotometer (Bruker Optics, Ettlingen, Germany) with KBr pellets. 1D and 2D NMR spectra were carried out on Bruker AM-400, DRX-500, or AVANCE III-600 spectrometers with TMS as an internal standard (Bruker, Karlsruhe, Germany). ESI-MS were run on an Agilent 6530 Q spectrometer (Agilent, Palo Alto, CA, USA). HR-EI-MS were measured using a Waters Auto Premier P776 spectrometer (Waters, Milford, MA, USA). Column chromatography (CC) was performed using MCI gel (CHP 20P, 75–150 μm; Mitsubishi Chemical Corporation, Tokyo, Japan) and silica gel (100–200 or 200–300 mesh; Qingdao Haiyang Chemical Co., Ltd, Qingdao, China). Thin-layer chromatography (TLC) was conducted on silica gel plates GF$_{254}$ (Qingdao Haiyang Chemical Co., Ltd, Qingdao, China). Fractions were monitored by TLC using various solvent systems, and spots were visualized by spraying improved Dragendorff’s reagent to the silica gel plates.
3.2. Plant material

The whole plants of *Vinca major* were collected in Kunming Botanical Garden, Yunnan Province, China, in September 2012 and identified by Prof. Xiao Cheng, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (120925) has been deposited in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, China.

3.3. Extraction and isolation

The air-dried and powdered whole plants of *V. major* (20 kg) were extracted with 90% EtOH (3 × 30 L, 3 d, each) at r.t. and then concentrated under vacuum to yield an extract (1050 g), which was partitioned between EtOAc and 5% HCl solution. The acidic water-soluble material was adjusted to pH 9–10 with 10% ammonia solution and then extracted with CHCl3 to give an alkaloidal extract (100 g). The alkaloidal extract was subjected to medium pressure liquid chromatography (MPLC) over MCI (1 kg) and eluted with MeOH-H2O (0, 20, 30, 40, 50, 60, 70, 80, 90, and 100%, 4L for each gradient) to yield five subfractions A-E. Fraction B (6.5 g) was separated by silica gel CC (CHCl3-MeOH, 19:1–10:1) to afford compound 2 (5 mg) and a mixture (200 mg). The mixture was further purified by HPLC eluting with 28% MeOH-H2O to provide compound 1 (46 mg, flow rate 3 ml/min; UV detection at 254 nm; tR 21.5 min). Fraction C (34.6 g) was subjected to silica gel CC (CHCl3-MeOH, 50:1–10:1) to yield compounds 3 (22 mg), 6 (60 mg), and 8 (30 mg). Compound 7 (20 mg) was obtained from fraction D (31.6 g) over silica gel CC (petroleum ether-Me2CO, 8:1–1:1). Fraction E (16.4 g) was applied to silica gel CC eluting with petroleum ether-Me2CO 15:1–1:1 to afford compounds 4 (34 mg) and 5 (54 mg).

3.3.1. 19-Hydroxyl-10-methoxy-19,20-dihydrovinorine (1)

Colorless oil. [α]D20 + 4.23 (c 0.20, MeOH). UV (MeOH) λmax (log ε): 279 (3.69), 222 (3.94), 204 (3.91) nm. IR (KBr) νmax: 3440, 2933, 1678, 1472, 1204, 1032, 722 cm−1. For 1H and 13C NMR spectral data (CD3OD), see Table 1. ESI-MS (pos.): m/z 383 [M+H]+; HR-EI-MS: m/z 382.1894 [M]+ (calcd for C22H26N2O4, 424.1998).

3.3.2. 19-O-acetyl-10-methoxy-19,20-dihydrovinorine (2)

Colorless oil. [α]D20 + 15.46 (c 0.20, MeOH). UV (MeOH) λmax (log ε): 279 (3.86), 222 (4.14), 203 (4.11) nm. IR (KBr) νmax: 2931, 1740, 1469, 1231, 1034, 819 cm−1. For 1H and 13C NMR spectral data (CD3OD), see Table 1. ESI-MS (pos.): m/z 383 [M+H]+; HR-EI-MS: m/z 382.1894 [M]+ (calcd for C22H26N2O4, 424.1998).

3.3.3. 19,21α-Dihydroxyl-10-methoxy-19,20-dihydrovinorine (3)

Colorless oil. [α]D20 + 35.24 (c 0.21, MeOH). UV (MeOH) λmax (log ε): 279 (3.85), 222 (4.12), 204 (4.11) nm. IR (KBr) νmax: 3425, 2930, 1741, 1468, 1225, 1033, 818 cm−1. For 1H and 13C NMR spectral data (CD3OD), see Table 1. ESI-MS (pos.): m/z 399 [M+H]+; HR-EI-MS: m/z 398.1834 [M]+ (calcd for C22H26N2O5, 398.1842).
3.4. Cytotoxicity assay

Five human cancer cell lines, HL-60 (human myeloid leukemia), SMMC-7721 (hepatocellular carcinoma), A-549 (lung cancer), MCF-7 (breast cancer), and SW480 (colon cancer), were used in the cytotoxic assay. All the cells were cultured in RPMI-1640 or DMEM medium (Hyclone, Logan, Utah, USA), supplemented with 10% fetal bovine serum (Hyclone, USA) in 5% CO₂ at 37 °C. The assays were performed according to the MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) method in 96-well microplates [16]. Briefly, 100 µl of adherent cells was seeded into each well of 96-well cell culture plates and allowed to adhere for 12 h before drug addition, while suspended cells were seeded just before drug addition with an initial density of 1 × 10⁵ cells/ml. Each tumor cell line was exposed to the test compound at concentrations of 0.064, 0.32, 1.6, 8, and 40 µM in triplicates for 48 h, with cisplatin (Sigma-Aldrich, St Louis, USA) as a positive control. After each compound treatment, cell viability was detected and a cell growth curve was graphed. IC₅₀ values were calculated by Reed and Muench’s method [17].

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