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# Indole and carbazole alkaloids from *Glycosmis montana* with weak anti-HIV and cytotoxic activities

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**Abstract**

A diprenylated indole, (*E*)-3-(3-hydroxymethyl-2-butenyl)-7-(3-methyl-2-butenyl)-1H-indole (**1**), and six known carbazole alkaloids were isolated from the twigs and leaves of *Glycosmis montana* Pierre (Rutaceae). Their structures were determined on the basis of analysis of spectral evidence including 1D and 2D NMR and MS. The alkaloids (**1–3**) exhibited weak to moderate take in vitro inhibitory activity against HIV replication in C8166 cells, and they (as well as carbalexine A and B) had cytotoxic activity against the human leukaemia cell line CCRF-CEM.


*Keywords:* *Glycosmis montana*; Rutaceae; Diprenylated indole; Carbazole; Anti-HIV; C8166; Cytotoxicity; Leukaemia

## 1. Introduction

The plants of the genus *Glycosmis* (*G.*), growing naturally throughout the India–Malayan region, are shrubs belonging to the plant family Rutaceae. The genus is known to be a rich source of various types of alkaloids. *Glycosmis montana* Pierre is one of 11 species that grow in China (Huang, 1997). To the best of our knowledge, no medicinal use or description of its chemical components have been reported for any part of the shrub. In the course of our search for new bioactive lead compounds from Rutaceaous plants, we have investigated the chemical constituents of the twigs and leaves of *G. montana*. As a result, one new (**1**) and six known (**2–7**) alkaloids were obtained. The present paper deals with the isolation, structure elucidation and in vitro anti-HIV activity of three alkaloids (**1–3**) and cytotoxic activity of five alkaloids (**1–5**) against leukaemia cells.

## 2. Results and discussion

Alkaloid 1 had the molecular formula $C_{18}H_{23}NO$ as recorded from its high resolution mass spectrum, and UV spectrum $\lambda_{max}^{MeOH}$ 292, 282, 275 and 226 nm was characteristic of a typical indole chromophore (Shoji et al., 1988; Riemer et al., 1997). A proton signal at $\delta$ 9.77, exchangeable with $D_2O$, was determined as a NH group since it had no HMQC correlation, which supported the indole skeleton. The $^{13}C$ NMR spectrum (Table 1)

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Table 1
**1** NMR spectroscopic data[a] for **1**

| Position | $\delta_H$ (*J* in Hz) | $\delta_C$ (*m*) | COSY | HMBC (H→C) |
|---|---|---|---|---|
| 1 | 9.77 *br s* | – | – | – |
| 2 | 7.04 *br s* | 122.4 (*d*) | – | 7a, 3, 1′ |
| 3 | – | 115.7 (*s*) | – | – |
| 3a | – | 128.5 (*s*) | – | – |
| 4 | 7.38 *d* (7.5) | 117.3 (*d*) | 5 | 3, 6, 3a, 7a |
| 5 | 6.94 *dd* (7.5,7.2) | 119.6 (*d*) | 4,6 | 7, 3a, 6 |
| 6 | 6.92 *d* (7.2) | 121.3 (*d*) | 5 | 1″, 4, 7a |
| 7 | – | 125.0 (*s*) | – | – |
| 7a | – | 136.6 (*s*) | – | – |
| 1′ | 3.47 *d* (7.2) | 24.3 (*t*) | 2′ | 3, 2, 3a, 2′ |
| 2′ | 5.69 (*m*) | 124.2 (*d*) | 1′ | 5′, 1′,4′,3 |
| 3′ | – | 136.2 (*s*) | – | – |
| 4′ | 3.97 *br s* | 68.3 (*t*) | – | 5′, 2′ |
| 5′ | 1.73*s* | 13.7 (*q*) | – | 2′, 4′ |
| 1″ | 3.56 *d* (7.1) | 29.6 (*t*) | 2″ | 7, 3″, 7a, 2″ |
| 2″ | 5.43 *m* | 123.2 (*d*) | 1″ | 5″, 4″, 1″ |
| 3″ | – | 133.1 (*s*) | – | – |
| 4″ | 1.74*s* | 25.8 (*q*) | – | 2″ |
| 5″ | 1.78*s* | 17.9(*q*) | – | 2″ |

[a] Measured in $CD_3COCD_3$ at 400 ($^1H$) and 100 Hz ($^{13}C$).

displayed 18 carbon signals, of which three were $CH_3$, three $CH_2$ (one of them bearing oxygen), six CH and six quaternary carbons. The $^1H$ NMR spectrum (Table 1) exhibited signals for four aromatic methine protons, of which one was a singlet for the pyrrole ring proton and three were mutually coupled protons at $\delta$ 7.38 (*d*, $J = 7.5$ Hz), 6.94 (*dd*, $J = 7.5$, 7.2 Hz) and 6.92 (*d*, $J = 7.2$ Hz) for benzene protons. Two sets of $-CH_2CH=$ signals, three singlet methyls and one oxymethylene group suggested the presence of two prenyl side chains. The structural fragments **1a**, **1b**, and **1c** (Fig. 1) were determined by interpretation of the COSY and HMBC spectrum (Table 1). Cross-peaks from three benzene protons and one pyrrole ring proton to their neighbouring carbons indicated substructure **1a**. Correlations of signals at $\delta$ 3.47 (H-1′), 5.69 (H-2′), 3.97 (H-4′), 1.73 (H-5′) also suggested the existence of substructure **1b**. Furthermore, correlations of signals at $\delta$ 3.56 (H-1″), 5.43 (H-2″), 1.74 (H-4″), 1.78 (H-5″) implied substructure **1c**, the total analysis of which led to compound **1**. The (*E*) configuration at the C-2′=C-3′ double bond was established by the ROESY (Fig. 1) correlation between H-2′ and H-4′. Hence, compound **1** was (*E*)-3-(3-hydroxymethyl-2-butenyl)-7-(3-methyl-2-butenyl)-1H-indole.

The structural determination of known compounds **2–7** was made based on comparison of the NMR spectroscopic data with the literature data for glybomine B (**2**) (Ito et al., 2004), glycoborinine (**3**) (Chakravarty et al., 1999), carbalexine B (**4**); carbalexine A (**5**); carbalexine C (**6**) (Pacher et al., 2001) and 2-hydroxy-3-methyl-9 H-carbazole (**7**) (Bhattacharyya et al., 1986).

A number of 3,5- and 3,6-diprenylated indoles, the hexalobins, have been reported for the Annonaceae genera *Uvaria* (Achenbach and Raffelsberger, 1979), *Hexalobus* (Achenbach and Löwel, 1995) and *Isolona* (Achenbach et al., 1984). However, there are only two examples of 3,7-diprenylated indoles reported in the Rutaceae plants *Esenbeckia leiocarpa* (Delle Monache et al., 1989) and *Glycosmis trichanthera* (Vajrodaya et al., 1998). Hence, this type of indole might be of chemotaxonomic significance for the Rutaceae.

Siamenol, a penylated carbazole alkaloid isolated from *Murraya siamensis*, was reported to have anti-HIV activity ($EC_{50} = 2.6$ μg/ml), in the XTT-tetrazolium assay (Meragelman et al., 2000). Therefore, three preny-

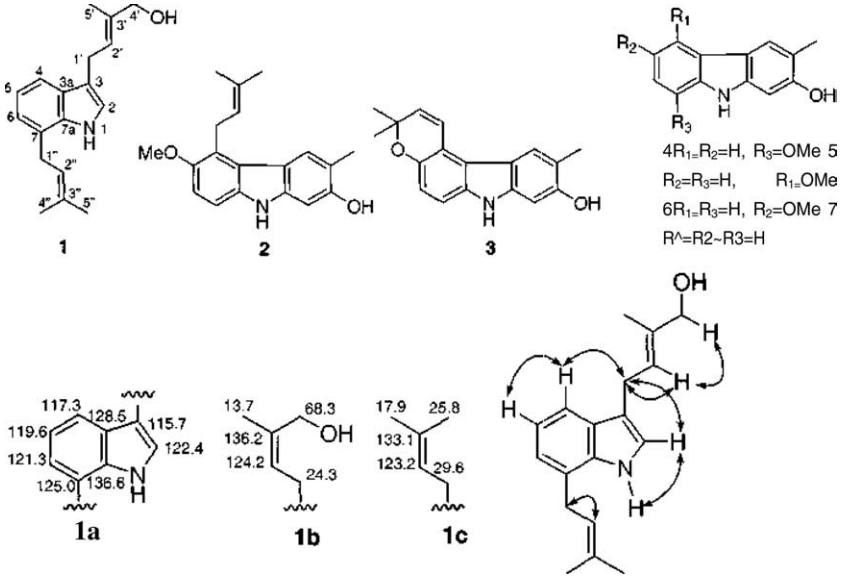


Fig. 1. Structural fragments of **1** and selected ROESY correlations.

Table 2
Anti-HIV activity of compounds **1**–**3**

| | $CC_{50}$ (μg/ml) | $IC_{50}$ (μg/ml) | SI |
|---|---|---|---|
| **1** | 13.66 | 1.17 | 11.68 |
| **2** | 13.62 | 9.73 | 1.40 |
| **3** | 20.69 | 4.47 | 4.63 |
| AZT | 794.2 | 0.131 | 6063 |

Anti-HIV activity was tested by inhibition assay for the cytopathic effects of HIV-1, as described in Section 3.

lated alkaloids (**1–3**) isolated from *G. montana* were tested for in vitro inhibitory effects against HIV replication in C8166 cells. The data are listed in Table 2. Carbazole alkaloids **2** and **3** demonstrated weak to moderate anti-HIV activity with an $IC_{50}$ value of 9.73 μg/ml for **2** and 4.47 μg/ml for **3**. The associated selectivity index (SI) values of 1.40 for **2** and 4.63 for **3**, however, were considered unfavourable for their further development. The new diprenylated indole alkaloid **1** exhibited the most potent anti-HIV activity with an $IC_{50}$ value of 1.17 μg/ml and SI value of 11.68. This is the first report of anti-HIV activity of this type of indole alkaloid. It is thus suggested that diprenylated indole alkaloids might be candidates for further study as potential anti-HIV agents. Applying a proliferation assay, we found $IC_{50}$ values of 5.1 (19.0) (**1**), 5.2 (17.6) (**2**), 2.1 (7.5) (**3**), 2.6 (11.5) (**4**) and 4.1 (18.1) μg/ml (μM) (**5**), respectively. As control, we used paclitexel, a natural product from *Taxus brevifolia*, and teniposide a semisynthetic derivative of podophyllotoxin from *Podophyllum peltatum*. The $IC_{50}$ values for CCRF-CEM cells were 96 and 4 nM, respectively. Hence, the five alkaloids showed similar cytotoxic activities, indicating that introduction of prenyl group did not significantly change the cytotoxic activity of these alkaloids. The three prenylated alkaloids exhibited higher cytotoxic activity against the human leukaemia cell line CCRF-CEM than against normal C8166 cells, suggesting possible selectivity against tumour cells. A similar phenomenon was observed for prenylated flavanones (Shirataki et al., 2001) isolated from *Sophora tomentosa* and *Sophora moorcroftiana* (Leguminosae).

## 3. Experimental

### 3.1. General

$^1H$, $^{13}C$, and 2D NMR spectra were recorded on a Bruker AM 400 NMR and a DRX-500 spectrometer, respectively, with TMS as internal standard. MS data were obtained on a VG AutoSpec 3000 spectrometers. UV spectra were obtained on a Shimadzu double-beam 210A spectrophotometer. The IR (KBr) spectra were recorded on a Bio-Rad FTS-135 spectrometer. Silica gel (200–300 mesh) for column chromatography and $GF_{254}$ for TLC were obtained from the Qindao Marine Chemical Factory, Qindao, PR China. HPLC separations were performed on a HP 1100 apparatus equipped with Diode array UV detector and XTERRA® C18 (Waters, 10 μm, 15 × 200 mm) column.

### 3.2. Plant material

The twigs and leaves (4 kg) of *G. montana* were collected in Xishuangbanna, Yunnan, China, in March 2003. The plant material was identified by Prof. De-Ding Tao, and a voucher specimen (BN20030408) was deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

### 3.3. Extraction and isolation

The dried powdered plant material (4 kg) was extracted by percolation with methanol (20 L) at room temperature for three weeks. The combined extracts were concentrated under reduced pressure to yield a dark green gum (420 g) which was suspended in water and partitioned with $CHCl_3$, EtOAc and *n*-BuOH, respectively. The chloroform soluble part (130 g) was subjected to silica gel column chromatography (CC) (64 cm × 10 cm) eluting with a gradient mixture of petroleum ether–EtOAc giving nineteen fractions. Fraction 12 (5.8 g) was then applied to silica gel CC, eluted with a petroleum ether–acetone gradient from (9:1 to 1:1) as eluant to give four subfractions (A–D). Fraction B (2.1 g) was repeatedly subjected to CC on silica gel, eluted with petroleum ether–acetone (8:2), to give **3** (200 mg) and **4** (140 mg). Fraction D (1.1 g) was then applied to silica gel CC, eluted with chloroform–acetone (9:1) to give **5** (50 mg) and **6** (6 mg). Fraction 13 (3.9 g) was further purified by silica gel CC, using chloroform–acetone (9:1) as eluent to afford **7** (3 mg), as well as mixture of compounds **1** and **2**. The mixture was further separated by RP-HPLC with MeOH–$H_2O$ (65:35, flow rate 10 ml/min) to yield 60 mg **1** ($t_R$ 25 min) and 40 mg **2** ($t_R$ 28 min).

#### 3.3.1. (*E*)-3-(3-Hydroxymethyl-2-butenyl)-7-(3-methyl-2-butenyl)-1*H*-indole (1)

Colourless oil; UV (MeOH) $\lambda_{max}$ nm: 226, 275 (sh), 282, 292 (sh); IR $\nu_{max}$ (KBr) $cm^{-1}$: 3420, 3054, 2970, 2915, 2857, 1494, 1437, 1379, 1351, 1282, 1221, 1102, 1057, 1014, 991, 924, 846; for $^1H$ and $^{13}C$ NMR spectroscopic data, see Table 1; EIMS: *m/z* 269 $[M]^+$, 238, 198, 194, 185, 182, 170, 167, 154, 142, 130, 115, 69; positive ion HRFAB-MS: $[M + 1]^+$, found: 270.1868; calc. for $C_{16}H_{23}NO$: 270.1857.

### 3.4. Biological assays

#### 3.4.1. Anti-HIV Assay

Test samples were first dissolved in DMSO and fivefold diluted with it into six concentrations: 200, 40, 8, 1.6, 0.32, and 0.064 μg/ml. The C8166 cells were cultured in complete medium (RPMI 1640 with 10% fetal calf serum supplemented with L-glutamine) at 5% $CO_2$ and 37 °C at all times. An aliquot of C8166 cells ($3 \times 10^5$/ml) was cultured only in medium for toxicity assay, while another aliquot was infected with HIV-1 (IIIB isolate) at a multiplicity of infection (MOI) at 0.015 for the cytopathic effect inhibition assay. All were incubated under identical conditions. After 4 h incubation, cells were washed thoroughly with fresh medium to remove unadsorbed virions and resuspended at $3 \times 10^4$ cells/ml. Then, 100 μl of cells seeded on a microtiter plate containing 100 μl various concentrations of the test compounds (positive infected control) or culture medium (negative-control drug), AZT was used as a positive-control drug. After incubation for 3 days, toxicity and cytopathic effects were measured.

The cellular toxicity of compounds on C8166 cells was assessed by MTT colorimetric assay as described previously (Zheng et al., 1995). Discarding 100 μl supernatant, MTT reagent was added and incubated for 4 h, and 100 μl of 50% DMF-10% SDS was added. After the formazum was dissolved completely, the plates were read on a Bio-Tek Elx 800 ELISA reader at 595/630 nm. Cell viability (% of control) was calculated by measuring absorbance values. $CC_{50}$ was defined as the concentration of the sample at which the absorbance value was reduced by 50%.

The cytopathic effect was measured by counting the number of syncytia (multinucleated giant cell) in each well under an inverted microscope (Zheng et al., 1999). The percentage inhibition of syncytial cell formation was calculated by percentage of syncytial cell numbers in compound treated cultures to that of infected control culture. The concentration of the antiviral sample reducing HIV-1 replication by 50% ($IC_{50}$) was determined from the dose response curve. The selectivity index (SI) was calculated from the ratio of $CC_{50}/IC_{50}$.

#### 3.4.2. Cytotoxicity assay

The in vitro response to drugs was evaluated by means of a growth inhibition assay as described (Efferth et al., 2003). Aliquots of $5 \times 10^4$ cells/ml were seeded in 24-well plates and drugs were added immediately at different concentrations, drugs were used in a dose range from 0.3 to 10 μg/ml to allow calculation of inhibition concentration 50% ($IC_{50}$) values. Paclitexel and teniposide were used as positive-control drugs. Cells were counted 7 days after treatment with the drugs. The resulting growth data represent the net outcome of cell proliferation and cell death. Vehicle controls were included for DMSO as solvent.

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