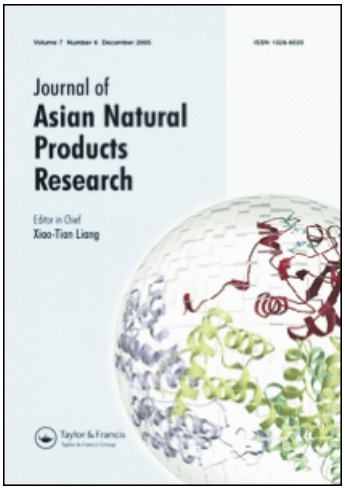


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# Alkaloids from *Clematis manshurica* Rupr.

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# Alkaloids from *Clematis manshurica* Rupr.

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Three new alkaloids together with two known compounds have been isolated from the roots of *Clematis manshurica*. On the basis of their spectroscopic and chemical evidence, the new compounds were elucidated as methyl 7-ethoxy-3-indolecarbonate (**2**), methyl 7-*O*-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranosyl 3-indolecarbonate (**3**) and α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranosyl 3-indolecarbonate (**4**).

*Keywords*: *Clematis manshurica* Rupr; Alkaloid; Methyl 7-ethoxy-3-indolecarbonate; Methyl 7-*O*-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranosyl 3-indolecarbonate; α-L-Rhamnopyranos-yl-(1 → 6)-β-D-glucopyranosyl 3-indolecarbonate

## 1. Introduction

*Clematis manshurica* Rupr., the roots and rhizomes of the plant named Weilingxian in traditional Chinese medicine (TCM), is widely distributed in the northeast of China. It is popularly used as an anti-inflammatory, anti-tumour, analgesic agent in TCM. Previous investigations revealed that the genus of *Clematis* possessed rich saponins and trace alkaloids such as magnoflorine, corytuberine [1], clemain and choline [2]. However, no alkaloids have been isolated from *C. manshurica*. Our investigation of the chemical constituents of *C. manshurica* yielded three new indole-type alkaloids and two known compounds. Here we report the isolation and structural elucidation of the alkaloids obtained from the plant.

## 2. Results and discussion

The 95% ethanol extract of the roots of *C. manshurica* was treated as described in the experimental section. The ethyl acetate and *n*-butanol extract were applied to column

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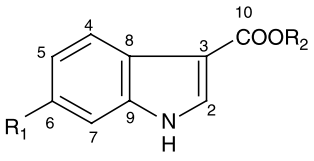
chromatography using silica gel, Sephadex LH-20 and $C_{18}$ HPLC-ODS, respectively, yielding compounds **1** and **2** from the ethyl acetate extract and compounds **3**, **4** and **5** from the *n*-butanol extract. Comparing with the data reported in the literature, compound **1** was identified as methyl 7-methoxyl 3-indolecarbonate [3], and compound **5** was determined as magnoflorine [4]. The new compounds **2**–**4** were elucidated as methyl 7-ethoxy-3-indolecarbonate (**2**), methyl 7-*O*-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranosyl 3-indolecarbonate (**3**) and α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranosyl 3-indolecarbonate (**4**) by analysing their spectroscopic evidence (figure 1).

Compound **1** was isolated as amorphous white powder. The ESI-MS spectrum showed the molecular ion peak at *m/z* 205. The $^1$H NMR spectrum indicated the presence of an ABX system of protons at $\delta$ 8.06 (1H, d, $J = 9.0$ Hz), 6.95 (1H, dd, $J = 2.4$, 9.0 Hz), 6.89 (1H, d, $J = 2.4$ Hz), a singlet aromatic proton at $\delta$ 7.82 (1H, s), two methoxy groups at $\delta$ 3.86 (3H, s) and 3.91 (3H, s). Its $^{13}$C NMR spectrum exhibited eight aromatic carbons, an ester carbonyl carbon ($\delta$ 165.67) and two methoxy carbons ($\delta$ 51.05, 55.61). Comparing with the NMR data reported in the literature [3], compound **1** was identified as methyl 7-methoxy-3-indolecarbonate, which was isolated from natural products for the first time.

Compound **2** was isolated as a white powder. The ESI-MS spectrum showed a molecular ion peak at *m/z* 219. The positive mode HRFAB-MS showed the presence of a molecular ion peak at *m/z* 219.0892, in accordance with an empirical molecular formula of $C_{12}H_{13}O_3N$, which was supported by the $^{13}$C NMR spectroscopic data. The NMR spectral data resembled very closely compound **1**, which suggested that compound **2** had a similar molecular skeleton to **1**. The only difference was that a methoxy group (C-6) in **1** was substituted by an ethoxy in **2**. In the HMBC spectrum of **2**, the cross-peak between the protons at $\delta$ 4.42 (2H, q, $J = 7.2$ Hz) and the carbon at 157.00 (C-6) indicated that the ethoxyl group was linked to C-6. Accordingly, the structure of compound **2** was elucidated as methyl 7-ethoxy-3-indolecarbonate.

Compound **3** was obtained as a light brown gummy material. The positive-ion HRFAB-MS showed the molecular ion $[M + Na]^+$ at *m/z* 522.1631, corresponding to the formula $C_{22}H_{29}O_{12}N$. Its IR spectrum showed the absorption of ester carbonyl group at 1695 cm$^{-1}$ and aromatic ring absorptions at 1620 and 1523 cm$^{-1}$. The $^1$H NMR spectrum indicated the presence of an ABX system of protons at $\delta$ 7.88 (1H, d, $J = 8.0$ Hz), 6.99 (1H, dd, $J = 2.0$, 8.0 Hz), 7.14 (1H, d, $J = 2.0$ Hz), a singlet aromatic proton at $\delta$ 7.83 (1H, s) and an ester




| | $R_1$ | $R_2$ |
|---|---|---|
| **1** | $OCH_3$ | $CH_3$ |
| **2** | $OCH_2CH_3$ | $CH_3$ |
| **3** | α-L-Rha (1→6)-β-D-Glu-*O* | $CH_3$ |
| **4** | H | α-L-Rha (1→6)-β-D-Glu-*O* |

Figure 1. Structures of compounds **1**–**4**.

methoxy at $\delta$ 3.82 (3H, s). Its $^{13}$C NMR spectrum exhibited eight aromatic carbons and an ester carbonyl carbon ($\delta$ 167.39). All investigations suggested that compound **3** had a similar molecular skeleton to **1**, however, a methoxy group in **1** disappeared in **3**, but two hexoses were indicated. Further analysis of the spectral data of **3** suggested the hexoses might be a glucosyl moiety and a rhamnosyl moiety, the anomeric carbons resonating at $\delta$ 103.32 and $\delta$ 102.46. The presence of the downfield $CH_2$ signal at $\delta$ 68.53 (C-6′) in the $^{13}$C NMR spectrum indicated that the rhamnosyl unit was probably linked to the C-6 site of the glucosyl unit. Two anomeric protons were resonating at $\delta$ 4.80 (1H, d, $J = 7.5$ Hz) and 4.69 (1H, br.s), respectively. The large coupling constant ($J = 7.5$ Hz) suggested that the glucosyl moiety existed in β orientation. The protons at $\delta$ 1.18 (3H, s) in the $^1$H NMR spectrum and carbon at $\delta$ 17.93 in the $^{13}$C NMR spectrum was attributed to the presence of the methyl group of rhamnosyl moiety.

In the HMBC spectrum, the anomeric proton at $\delta$ 4.80 showed long-range correlation with the carbon at $\delta$ 156.00 (C-6), suggesting the attachment of the glucosyl moiety at C-6. The anomeric proton at $\delta$ 4.69 indicated interaction with the carbon at $\delta$ 68.53 (C-6 of the glucose), confirming that the rhamnosyl unit was linked to C-6 of the glucosyl unit. The sugars were confirmed by acid hydrolysis and comparison with authentic sugars on TLC. Consequently, the structure of **3** was elucidated as methyl 7-*O*-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranosyl 3-indolecarbonate.

Compound **4** was isolated as a light brown gummy material. Its molecular formula was assigned as $C_{21}H_{27}O_{11}N$ by the molecular ion peak $[M + Na]^+$ at $m/z$ 492.1488 in the HRFAB-MS spectrum. The spectral data of **4** were very similar to those of **3**. In the $^{13}$C NMR spectrum of **4**, the signal of the ester methoxy disappeared and the anomeric carbon of the glucosyl unit was upfield at $\delta$ 95.28, suggesting that the attachment of the sugar moiety was at C-10. The presence of a downfield $CH_2$ signal at $\delta$ 67.82 indicated that the rhamnosyl unit was connected at C-6 of the glucosyl unit. All these conclusions were confirmed by HMBC experiments. The $^1$H NMR spectrum showed four aromatic protons for an *ortho*-disubstituted aromatic ring at $\delta$ 8.11 (1H, m), 7.20 (2H, m), 7.45 (1H, m) and a singlet signal at 8.06 (1H, s). Compared with **3**, compound **4** was identified as α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranosyl 3-indolecarbonate.

## 3. Experimental

### *3.1 General experimental procedures*

Melting points were determined on an X-4 digital micro-melting point apparatus and are uncorrected. NMR spectra were recorded in $CD_3OD$ and $CDCl_3$ using Inova 300 and Inova 500 MHz NMR spectrometers with tetramethylsilane as internal standard. HRFAB-MS was measured on an Autospec-UltimaETOF spectrometer in positive ion mode. Optical rotations were recorded on a Perkin–Elmer 243B digital polarimeter. UV spectra were obtained from TU-1901 spectrometer. IR spectra were recorded on an AVATER-360 spectrometer. ESI-MS spectra were measured on a QSTAR mass spectrometer. PHPLC was carried out on an ODS column (Alltech 250 × 10 mm i.d., 5 μm) with a Waters 2996 photodiode array detector. For CC, silica gel (200–300 mesh, Qingdao Mar. Chem. Ind. Co. Ltd.), Sephadex LH-20 gel (Pharmacia) and D101 porous polymer resin (Tianjin Chem. Ind. Co. Ltd.) were used.

### 3.2 Plant material

*Clematis manshurica* Rupr. was collected in August 2002 from Heilongjiang province, northeast China. The identification of the plant was performed by Professor Pengfei Tu, Peking University. A voucher specimen is kept in the herbarium of Peking University Modern Research Centre for Traditional Chinese Medicine (CM200208).

### 3.3 Extraction and isolation

The dried roots (15 kg) of *C. manshurica* were extracted with boiling 95% ethanol three times. After removal of the solvent under reduced pressure at 60°C, the residue (1.7 kg) was suspended in water and defatted with petroleum ether. The aqueous layer was further extracted with ethyl acetate and *n*-butanol successively. A portion of ethyl acetate extract (40 g) was subjected to silica gel (1 kg) column chromatography and eluted with $CHCl_3$/MeOH in gradient mode; the fractions 8–10 (500 ml/Fr., eluted with $CHCl_3$/MeOH 50:1) were isolated by silica gel CC (solvent: $CHCl_3$/MeOH 6:1–10:1) repeatedly and purified by PHPLC ($CH_3CN/H_2O = 45:55$, 2.5 ml/min) to yield compound **1** (5 mg) and compound **2** (6 mg). The *n*-butanol extract (250 g) was subjected to D101 porous polymer resin and eluted with MeOH/$H_2O$ in gradient mode. The fraction eluted by $H_2O$ (70 g) was treated with 75% ethanol, the precipitate filtered and the solvent evaporated under reduced pressure; the residue (20 g) was subjected to silica gel (500 g) column chromatography and eluted with $CHCl_3$/MeOH/$H_2O$ (65:35:10, the lower layer). Fractions 34–40 (30 ml/Fr.) were purified by PHPLC ($CH_3CN/H_2O = 10:90$, 2.5 ml/min), resulting in the yield of compound **5** (8 mg). The fraction eluted by 30% methanol (20 g) was subjected to Sephadex LH-20 and eluted with $H_2O$, The fractions 6–8 (50 ml/Fr.) were isolated by Sephadex LH-20 repeatedly and purified by PHPLC ($CH_3CN/H_2O = 15:85$, 2.5 ml/min) to yield compounds **3** (9 mg) and **4** (7 mg).

### 3.4 HPLC analysis of compounds 1 and 2 in the 70% acetone extract of C. manshurica

The dried roots (100 g) were extracted with 70% acetone three times. After evaporating the solvent under reduced pressure, the residue was defatted by petroleum ether and analysed by HPLC.

HPLC analysis was performed with Alltech column (250 × 10 mm i.d. 5 μm) at the column temperature of 25°C. The mobile phase composed of $CH_3CN/H_2O$ (45:55, v/v) was eluted at a flow rate of 2.5 ml/min. Elutes were monitored by a 2996 photodiode array detector at 275 nm. HPLC analysis indicated peaks with the same retention time as compounds **1** and **2**, which suggested that compounds **1** and **2** were naturally occurring compounds and not artefacts formed during extraction and separation.

### 3.5 Acid hydrolysis of compounds 3 and 4

Compounds **3**, **4** and the authentic sugars were dissolved in methanol and spotted on a silica gel plate, then the plate was fumigated by the steam of hydrochloric acid for 30 min; after this period, the plate was expanded by *n*-butanol/EtOAc/2-propanol/HOAc/$H_2O$ (7:20:12:7:6).

Table 1. NMR data of compounds **1**, **2** (in $CDCl_3$) and **3**, **4** (in $CD_3OD$).

| | ***1***[a] | | ***2***[a] | | ***3***[b] | | ***4***[b] | |
|---|---|---|---|---|---|---|---|---|
| *No.* | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ |
| 2 | 7.82 (1 H, s) | 129.91 | 7.81 (1 H, s) | 129.91 | 7.83 (1 H, s) | 132.98 | 8.06 (1 H, s) | 134.29 |
| 3 | | 108.78 | | 108.78 | | 108.44 | | 107.51 |
| 4 | 8.06 (1 H, d, $J = 9.0$ Hz) | 119.92 | 8.06 (1 H, d, $J = 9.0$ Hz) | 119.92 | 7.88 (1 H, d, $J = 8.0$ Hz) | 122.39 | 8.11 (1 H, m) | 122.06 |
| 5 | 6.95 (1 H, dd, $J = 2.4$, 9.0 Hz) | 111.80 | 6.95 (1 H, dd, $J = 2.4$, 9.0 Hz) | 111.80 | 6.99 (1 H, dd, $J = 2.0$,,8.0 Hz) | 114.35 | 7.20 (2 H, m) | 122.74 |
| 6 | | 157.06 | | 157.00 | | 156.00 | 7.20 (2H, m) | 123.90 |
| 7 | 6.89 (1H, d, $J = 2.4$ Hz) | 94.86 | 6.90 (1H, d, $J = 2.4$ Hz) | 94.87 | 7.14 (1H, d, $J = 2.0$ Hz) | 100.39 | 7.45 (1H, m) | 113.04 |
| 8 | | 122.13 | | 122.13 | | 122.73 | | 127.51 |
| 9 | | 136.86 | | 136.86 | | 138.48 | | 138.20 |
| 10 | | 165.67 | | 165.67 | | 167.39 | | 165.40 |
| Glu | | | | | | | | |
| 1′ | | | | | 4.80 (1H, d, $J = 7.5$ Hz) | 103.32 | 5.70 (1H, d, $J = 8.0$ Hz) | 95.28 |
| 2′ | | | | | 3.36 (1H, m) | 74.97 | 3.52 (1H, m) | 74.15 |
| 3′ | | | | | 3.41 (1H.m) | 78.01 | 3.49 (1H.m) | 78.21 |
| 4′ | | | | | 3.28 (1H.m) | 71.73 | 3.31 (1H.m) | 72.11 |
| 5′ | | | | | 3.54 (1H.m) | 77.01 | 3.58 (1H.m) | 77.72 |
| 6′ | | | | | 4.39 (1H, dd, $J = 5.0$, 11.5 Hz) | 68.53 | 3.99 (1H, dd, $J = 2.0$, 11.5 Hz) | 67.82 |
| | | | | | 3.62 (1H, dd, $J = 5.0$, 11.5 Hz) | | 3.65 (1H, dd, $J = 5.0$, 11.5 Hz) | |
| Rha | | | | | | | | |
| 1″ | | | | | 4.69 (1H, br. s) | 102.46 | 4.71 (1H, br. s) | 102.28 |
| 2″ | | | | | 3.78 (1H.m) | 71.17 | 3.89 (1H.m) | 71.27 |
| 3″ | | | | | 3.70 (1H.m) | 72.34 | 3.66 (1H.m) | 72.32 |
| 4″ | | | | | 3.42 (1H.m) | 74.08 | 3.40 (1H.m) | 74.01 |
| 5″ | | | | | 3.70 (1H.m) | 69.71 | 3.82 (1H.m) | 69.81 |
| 6″ | | | | | 1.18 (3H, d, $J = 6.0$ Hz)) | 17.93 | 1.19 (3H, d, $J = 6.0$ Hz)) | 17.95 |
| $OCH_3$ | 3.91 (3H, s) | 55.61 | | | | | | |
| $OCH_3$ | | | | | | | | |
| | 3.86 (3H, s) | 51.05 | 3.85 (3H, s) | 51.05 | 3.82 (3H, s) | 51.39 | | |
| $CH_2$ | | | 4.42 (2H, q, $J = 7.2$ Hz) | 59.77 | | | | |
| $CH_3$ | | | 1.42 (3H, t, $J = 7.2$ Hz) | 14.54 | | | | |

[a] Recorded on Inova 300 MHz NMR spectrometer.
[b] Recorded on Inova 500 MHz NMR spectrometer.

D-Glc and L-Rha were detected from compounds **3** and **4** by comparing with the authentic sugars. The $R_f$ of the D-Glc and L-Rha were 0.58 and 0.75, respectively.

Compound **1**: amorphous white powder; mp 163–165°C; UV (MeOH): $\lambda_{max}$ 322, 275, 228, 218 nm; ESI-MS *m/z*: 205 [$M^+$]; $^1$H NMR and $^{13}$C NMR spectral data: see table 1.

Compound **2**: amorphous white powder; mp 172–174°C; UV (MeOH): $\lambda_{max}$ 322, 275, 228, 218 nm; ESI-MS *m/z*: 219 ($M^+$); HRFAB-MS *m/z*: 219.0892 (calcd for $C_{12}H_{13}O_3N$, 219.0895); $^1$H NMR and $^{13}$C NMR spectral data: see table 1.

Compound **3**: light brown gum; mp 133–135°C; $[\alpha]_D^{20}$ −122.5 (*c* 2.0, MeOH), UV (MeOH): $\lambda_{max}$ 322, 279, 214, 192 nm; IR (KBr): $\nu_{max}$ 3382, 2927, 1695, 1620, 1523, 1435, 1383 $cm^{-1}$; HRFAB-MS *m/z*: 522.1631 $[M + Na]^+$ (calcd for $C_{22}H_{29}O_{12}N + Na$, 522.1612); $^1$H NMR and $^{13}$C NMR spectral data: see table 1.

Compound **4**: amorphous white powder; mp 130–132°C; $[\alpha]_D^{20}$ −121.0 (*c* 2.0, MeOH), UV (MeOH): $\lambda_{max}$ 322, 285, 228, 210 nm; IR (KBr): $\nu_{max}$ 3378, 2922, 1693, 1625, 1520, 1438, 1380 $cm^{-1}$; HRFAB-MS *m/z*: 492.1488 $[M + Na]^+$ (calcd for $C_{21}H_{29}O_{11}N + Na$, 492.1482); $^1$H NMR and $^{13}$C NMR spectral data: see table 1.



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