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# Polyacetylene glycosides from *Pratia nummularia* cultures

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**Abstract**

Two polyacetylene glycosides, lobetyol 9-*O*-glc$^{6}$-$^{1}$rha (pratialin-A) and lobetyol 9-*O*-glc$^{6}$-$^{1}$glc$^{6}$-$^{1}$glc (pratialin-B), were isolated from *Pratia nummularia* (Campanulaceae) callus and hairy root cultures and their chemical structures were determined by analysis of spectroscopic data. From the methanol extract of the hairy root cultures, together with the known polyacetylene constituents lobetyol, lobetyolin, and lobetyolinin, tryptophan was also isolated. This report is the first example of the isolation and structure elucidation of rutinoside (pratialin-A) and triglucoside (pratialin-B) derivatives of polyacetylene constituents.


*Keywords:* *Pratia nummularia*; Campanulaceae; Callus; Hairy root; *Agrobacterium*; Polyacetylene; Pratialin-A; Pratialin-B; Glycoside

## 1. Introduction

*Pratia nummularia* (Lam.) A. Br. et Asch (Campanulaceae) is a perennial prostrate plant distributed particularly in damp fields at high altitude (500–2300 m) in India, China, Malaysia, Myanmar, Thailand, South America, and Australia. The whole plant has been used as a traditional medicine for treatment of pus, contusion, cough and as an anti-inflammatory (Chiu, 1987). Flavonoids such as diosmin, linarin, apigenin 7-*O*-rutinoside, and luteolin 7-*O*-rutinoside, and a polyacetylene constituent lobetyolin have been isolated from whole plantlets of this species (Matsuura et al., 2000). Campanulaceous plants in the genera *Lobelia*, *Campanula*, *Platycodon*, *Wahlenbergia, Trachelium*, etc., have been shown to contain derivatives of a $C_{14}$-polyacetylene, lobetyol (Ishimaru et al., 1991, 1992). Tissue cultures of various species in Campanulaceae, especially *Agrobacterium rhizogenes*-mediated transformed root cultures (hairy roots), have produced large amounts of polyacetylene glycosides such as lobetyolin and lobetyolinin (Ishimaru et al., 1993, 1994, 1995, 1997, 1998; Tada et al., 1995a,b, 1996; Yamanaka et al., 1996; Ahn et al., 1996; Tanaka et al., 1996, 1999; Ando et al., 1997; Murakami et al., 1998). In the tissue culture and biochemical study of campanulaceous plants, two polyacetylene glycosides **1** and **2** were isolated from the callus and hairy root cultures of *P. nummularia*. Their chemical structures were elucidated through spectroscopic studies.

## 2. Results and discussion

Compound **1** was obtained as an off-white amorphous powder. Positive-ion fast atom bombardment mass spectroscopy (FAB-MS) of **1** gave a quasi-molecular ion peak at $m/z$ 565.2269 $[M+Na]^+$, which corresponded to the molecular formula $C_{26}H_{38}O_{12}$. The $^{1}$H NMR spectrum of **1** (Table 1) showed two anomeric protons at $\delta$ 4.41 ($J=7.6$ Hz) and $\delta$ 4.80 ($J=1.2$ Hz), together with one methyl ($\delta$ 1.81), two methine ($\delta$ 4.24 and $\delta$ 4.51), three methylene ($\delta$ 1.65, 2.19 and $\delta$ 3.58), and two pairs of *trans* coupled olefinic [$\delta$ 5.50, 5.95 ($J=15.3$ Hz) and $\delta$ 5.63, 6.37 ($J=15.0$ Hz)] proton signals which were closely correlated to those of polyacetylene constituents lobetyol, lobetyolin and lobetyolinin (Ishimaru et al., 1991, 1992). The $^{13}$C NMR spectrum of **1** (Table 2) also demonstrated the existence of two sugar moieties giving rise to 12 carbon signals (C-1′-6′ and C-1″-6″), whose chemical shifts showed the presence of glucopyranose and rhamnopyranose moieties.

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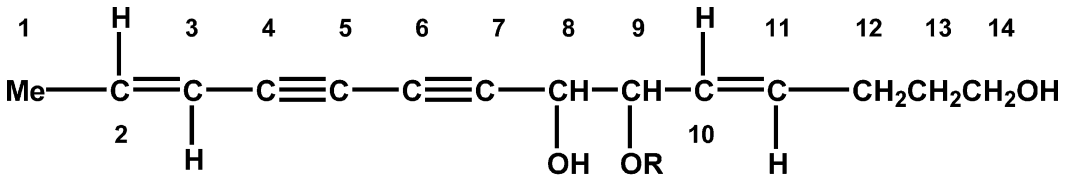


**lobetyol : R=H**

**lobetyolin : R=Glc**

**lobetyolinin : R=Glc$^{6}$-$^{1}$Glc**

**pratialin-A (1) : R=Glc$^{6}$-$^{1}$Rha**

**pratialin-B (2) : R=Glc$^{6}$-$^{1}$Glc$^{6}$-$^{1}$Glc**

Table 1
$^{1}$H NMR spectral data of compounds **1** and **2** ($Me_2CO$-$d_6$ + $D_2O$, 500 MHz, $\delta$ values)

| H | 1 | 2 |
|---|---|---|
| 1 | 1.81 3H, *dd* (1.8, 7.0) | 1.82 3H, *dd* (1.8, 7.0) |
| 2 | 6.37 1H, *dq* (15.0, 7.0) | 6.38 1H, *dq* (15.0, 7.0) |
| 3 | 5.63 1H, *ddd* (15.0, 0.9, 0.9) | 5.63 1H, *ddd* (15.0, 0.9, 0.9) |
| 8 | 4.51 1H, *d* (5.8) | 4.55 1H, *d* (5.8) |
| 9 | 4.24 1H, *dd* (5.8, 7.9) | 4.31 1H, *dd* (5.8, 7.9) |
| 10 | 5.50 1H, *ddd* (15.3, 7.9, 1.5) | 5.50 1H, *ddd* (15.3, 7.9, 1.5) |
| 11 | 5.95 1H, *dt* (15.3, 7.0) | 6.00 1H, *dt* (15.3, 7.0) |
| 12 | 2.19 2H, *br dd* (6.7, 13.1) | 2.18 2H, *br dd* (6.7, 13.1) |
| 13 | 1.65 2H, *quin* (6.7) | 1.67 2H, *quin* (6.7) |
| 14 | 3.58 2H, *t* (6.7) | 3.59 2H, *t* (6.7) |
| Glc-I | | |
| 1′ | 4.41 1H, *d* (7.6) | 4.46 1H, *d* (7.9) |
| 2′ | 3.39–3.43 1H, *m* | 3.25–3.60 1H, *m* |
| 3′ | 3.58 1H, *m* | 3.25–3.60 1H, *m* |
| 4′ | 3.39–3.43 1H, *m* | 3.25–3.60 1H, *m* |
| 5′ | 3.88 1H, *dd* (1.5, 3.4) | 3.25–3.60 1H, *m* |
| 6′ | 3.60 1H, *br d* (11.3) | 3.82 1H, *dd* (4.6, 11.6) |
| | 4.01 1H, *dd* (3.4, 11.3) | 4.19 1H, *br d* (11.6) |
| Glc-II | | |
| 1″ | – | 4.47 1H, *d* (7.9) |
| 2″ | – | 3.25–3.60 1H, *m* |
| 3″ | – | 3.25–3.60 1H, *m* |
| 4″ | – | 3.25–3.60 1H, *m* |
| 5″ | – | 3.25–3.60 1H, *m* |
| 6″ | – | 3.82 1H, *dd* (4.6, 11.6) |
| | 4.19 1H, *br d* (11.6) | |
| Glc-III | | |
| 1‴ | – | 4.48 1H, *d* (7.9) |
| 2‴ | – | 3.25–3.60 1H, *m* |
| 3‴ | – | 3.25–3.60 1H, *m* |
| 4‴ | – | 3.25–3.60 1H, *m* |
| 5‴ | – | 3.25–3.60 1H, *m* |
| 6‴ | – | 3.70 1H, *m*[a] |
| | | 3.88 1H, *d* (11.9) |
| Rha | | |
| 1″ | 4.80 1H, *d* (1.5) | – |
| 2″ | 3.60–3.70 1H, *m* | – |
| 3″ | 3.60–3.70 1H, *m* | – |
| 4″ | 3.60–3.70 1H, *m* | – |
| 5″ | 3.60–3.70 1H, *m* | – |
| 6″ | 1.24 3H, *d* (6.4) | – |

Coupling constants ($J$ in Hz) in parentheses.
[a] Overlapped with a HOD or $H_2O$ signal.

The low-field shifted signal of C-9 ($\delta$ 81.7), whose chemical shift was similar to those of lobetyolin and lobetyolinin, indicated the position of the glucose moiety to be at C-9 (Ishimaru et al., 1991, 1992). The low-field shift of the glucose C-6′ carbon signal ($\delta$ 67.9) also showed the location of rhamnose residue at this position (C-6′). The configuration of the anomeric carbons of the glucopyranose and rhamnopyranose units were concluded to be β and $\alpha$, respectively, from the $J$-values in the $^{1}$H NMR spectrum (Table 1). Therefore, **1** (pratialin-A) was identified as the rutinoside of lobetyol, lobetyol 9-*O*-glc$^{6}$-$^{1}$rha, and is the first example of a polyacetylene derivative having a rhamnose moiety in the molecule.

Compound **2**, an off-white amorphous powder, showed a quasi-molecular ion peak at $m/z$ 743.2742 $[M+Na]^{+}$, indicating the molecular formula of $C_{32}H_{48}O_{18}$ in the FAB-MS. The $^{1}$H NMR spectrum of **2** (Table 1) showed three anomeric protons at $\delta$ 4.46 ($J$ = 7.9 Hz), 4.47 ($J$ = 7.9 Hz), and $\delta$ 4.48 ($J$ = 7.9 Hz), which were suggested to arise from three glucopyranose moieties from the chemical shifts of the 18 carbohydrate carbon signals observed in the $^{13}$C NMR spectrum of **2** (Table 2). The $^{1}$H and $^{13}$C NMR spectra of **2** also revealed the presence of a similar structural sequence (C-1–C-14, lobetyol moiety) to that of **1**. Therefore, **2** was concluded to be triglucoside of lobetyol. Two low-field carbon signals [$\delta$ 69.3 (C-6′) and $\delta$ 69.6 (C-6″)] of glucopyranose moieties indicated the presence of a-glc$^{6}$-$^{1}$glc$^{6}$-$^{1}$glc structure, and the low-field shifted C-9 ($\delta$ 81.9) carbon signal indicated the triglucose moiety to be connected at this position. The configuration of three anomeric carbons of glucopyranose moieties were concluded to be β from the large coupling constants ($J$ = 7.9 Hz) in the $^{1}$H NMR spectrum (Table 1). This spectroscopic evidence indicated that **2** was the triglucoside of lobetyol, namely, lobetyol 9-*O*-glc$^{6}$-$^{1}$glc$^{6}$-$^{1}$glc. Compound **2** (pratialin-B) is the first example of a triglycoside derivative of a polyacetylene.

Table 2
$^{13}C$ NMR spectral data of compounds **1** and **2** ($Me_2CO$-$d_6$ + $D_2O$, 125 MHz, $\delta$ values)

| H | 1 | 2 |
|---|---|---|
| 1 | 18.7 | 18.7 |
| 2 | 145.2 | 145.3 |
| 3 | 110.1 | 109.9 |
| 4 | 82.0 | 81.7 |
| 5 | 77.8 | 77.9 |
| 6 | 72.6 | 72.5 |
| 7 | 70.4 | 70.5 |
| 8 | 66.2 | 66.0 |
| 9 | 81.7 | 81.9 |
| 10 | 125.9 | 125.8 |
| 11 | 138.3 | 138.4 |
| 12 | 29.5 | 29.3 |
| 13 | 32.8 | 32.4 |
| 14 | 61.6 | 61.5 |
| Glc-I | | |
| 1′ | 100.7 | 100.6 |
| 2′ | 74.4 | 74.1 |
| 3′ | 77.7 | 77.2 |
| 4′ | 71.4 | 70.9 |
| 5′ | 76.6 | 76.2 |
| 6′ | 67.9 | 69.3 |
| Glc-II | | |
| 1″ | – | 104.0 |
| 2″ | – | 74.3 |
| 3″ | – | 77.1 |
| 4″ | – | 70.8 |
| 5″ | – | 77.2 |
| 6″ | – | 69.6 |
| Glc-III | | |
| 1‴ | – | 104.2 |
| 2‴ | – | 74.3 |
| 3‴ | – | 77.1 |
| 4‴ | – | 70.6 |
| 5‴ | – | 77.2 |
| 6‴ | – | 62.1 |
| Rha | | |
| 1″ | 101.7 | – |
| 2″ | 71.6 | – |
| 3″ | 72.1 | – |
| 4″ | 73.6 | – |
| 5″ | 69.2 | – |
| 6″ | 18.1 | – |

## 3. Experimental

### 3.1. General

$^1H$ and $^{13}C$ NMR spectra were measured at 500 and 125 MHz, respectively, with references against the major deuterium signal of the solvents. All culture media containing 30 g/l sucrose were adjusted to pH 5.7 before autoclaving at 121 °C for 15 min.

### 3.2. Callus cultures

In vitro plantlets of *Pratia nummularia* (Lam.) A. Br. et Asch established in our previous study (Ishimaru and Matsuura, 2000) were cultured on half-strength Murashige and Skoog (1/2 MS) solid medium (Murashige and Skoog, 1962) for 8 weeks under illumination (16 h/day light condition, 60 μmol/$m^2$s, cool white fluorescent lamp: HITACHI FLR 40SW/M-G). The leaf segments (5×5 mm) cut from the in vitro plantlets were placed on 1/2 MS solid medium supplemented with 1.0 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.1 mg/l kinetin in the dark at 25±1 °C. The calli induced on the cut ends of the segment, were cut off and maintained on the similar culture medium above by subculturing at 8-week intervals in the dark.

### 3.3. Extraction and isolation from callus cultures

*P. mummularia* calli cultured for 8 weeks in the dark were lyophilized (58.8 g, dry weight), and extracted twice with 60% aqueous MeOH (900 ml), and 100% MeOH (500 ml). The extracts were mixed and concentrated in vacuo, and the resulting extract (16.3 g) was partitioned with $H_2O$ and EtOAc. The aqueous layer (14.3 g) was chromatographed over DIAION HP 20SS ion exchange resin (MITSUBISHI CHEMICAL, 3.5 cm i.d.×25.5 cm) with aqueous MeOH (20→40→100% MeOH), Sephadex LH-20 (Pharmacia, 2.5 cm i.d.×16.5 cm) with EtOH and Preparative $C_{18}$ 125Å (Waters Corporation, 2.5 cm i.d.×13.5 cm) with aqueous MeOH (0→40% MeOH) to afford a fraction containing the polyacetylene constituents. The fraction was subjected to preparative HPLC system with column: CAPCELL PAK C18 AG-120 (Shiseido Fine Chemical, 15 mm i.d.×250 mm), mobile phase: $H_2O$–MeOH (1:1), flow rate: 4 ml/min, detection (UV): 270 nm, column temperature: 40 °C. The fraction eluted at *Rt* 13.6 min was collected and further purified using the similar preparative HPLC system (CAPCELL PAK C18 AG-120, 15 mm i.d.×250 mm) eluted with $H_2O$–MeCN (85:25, v/v) to afford compound **1** (4.0 mg).

#### 3.3.1. Pratialin-A (*1*)

An off-white amorphous powder, $[\alpha]_D^{28}$ −62.4°(MeOH; *c* 0.29); for $^1H$ and $^{13}C$ NMR spectral data of **1**, see Tables 1 and 2; high-resolution FABMS positive FABMS *m*/*z* (rel. int.); 565.2269 (100), $[C_{26}H_{38}O_{12} + Na]^+$ (calcd 565.5688).

### 3.4. Hairy root cultures

*P. nummularia* hairy roots were derived by the infection with *Agrobacterium rhizogenes* strain ATCC 15834 and subcultured at 8-week intervals in phytohormone free 1/2 MS liquid medium in the dark on the rotary shaker (95 rpm) (Ishimaru and Matsuura, 2000).

### 3.5. Extraction and isolation from hairy root cultures

Lyophilized hairy roots (85.2 g, dry weight) were extracted with MeOH (600 ml×4) and 60% aqueous MeOH (800 ml). The extracts were mixed, concentrated in vacuo and applied to Sephadex LH-20 column (5 cm i.d.×36 cm) with aqueous MeOH (60→80→100% MeOH) to afford four fractions (Frs 1–4). Fr. 1 was subjected to preparative $C_{18}$ 125Å column chromatography (3 cm i.d. ×24 cm) with $H_2O$ containing increasing proportions of MeOH (0–80%) to afford six subfractions (Frs. 11–16). Frs. 14, 15, and 16 were purified with Shephadex LH-20 (EtOH) and/or silica-gel 60 (Merck, benzene– acetone, 3:1→3:2 v/v) cc to give lobetyolinin (34.6 mg from Fr. 14), lobetyolin (54.2 mg from Fr. 15), and lobetyol (43.3 mg from Fr. 16). Fr. 13 was subjected to preparative $C_{18}$ 125 Å column (3 cm i.d.×21 cm) with $H_2O$ containing increasing proportions of MeOH (0–60%) to afford a second fraction, which was purified by prep. HPLC system (column: CAPCELL PAK C18 AG-120, 15 mm i.d.×250 mm), mobile phase: $H_2O$–MeOH (1:1), flow rate: 4 ml/min, detection (UV): 270 nm, column temperature: 40 °C) to afford compound **2** (3.2 mg) and tryptophan (11 mg). The known polyacetylenes, lobetyol, lobetyolin, and lobetyolinin and the amino acid tryptophan were identified, respectively, by comparing their $^1H$ and $^{13}C$ NMR spectral data with those of the authentic standards.

#### 3.5.1. Pratialin-B (2)

An off-white amorphous powder, $[\alpha]_D^{28}$ −55.0°(MeOH; *c* 0.21); for $^1H$ and $^{13}C$ NMR data of **2**, see Tables 1 and 2; high-resolution positive FABMS *m*/*z* (rel. int.); 743.2742 (94.6), $[C_{32}H_{48}O_{18}+Na]^+$ (calcd 743.7102).

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