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# IRIDOID GLUCOSIDES WITH DIFFERENT ACYL MOIETIES FROM GLOBULARININ AND GLOBULARIMIN FROM LEAVES OF *PREMNA SUBSCANDENS*

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**Key Word Index**—*Premna subscandens*; verbenaceae; iridoid glucoside; globularinin; globularimin; iridoid glucoside 10-*O*-acyl ester.

**Abstract**—Five iridoid glucosides were isolated from leaves of *Premna subscandens*. Their structures were elucidated to be 4″-methoxy-E-, 4″-methoxy-Z- and 4″-hydroxy-E-globularinin and 4″-methoxy-E- and 4″-methoxy-Z-globularimin from spectroscopic evidence. © 1998 Elsevier Science Ltd. All rights reserved

## INTRODUCTION

In a previous paper, nine iridoid glucosyl 10-*O*-acyl catalpols and asystasioside Es were isolated from leaves of *Premna subscandens*, collected on Ishigaki Island, Okinawa [1]. Further extensive isolation work afforded a further five new iridoid glucosides which are also 10-*O*-acylated derivatives of known iridoid glucosides, namely 10-descinnamoyl globularinin and 10-descinnamoyl globularimin.

## RESULTS AND DISCUSSION

Compounds **1**–**5** were isolated from the 1-BuOH-soluble fraction of a MeOH extract of *P. subscandens* leaves (see Section 3, Experimental).

Compound **1** was shown to have the molecular formula $C_{25}H_{32}O_{13}$ by negative ion HR–FAB mass spectrometry. The IR spectrum showed the presence of hydroxyl groups (3325 $cm^{-1}$), a conjugated ester (1680 and 1625 $cm^{-1}$), and an aromatic ring (1598 and 1505 $cm^{-1}$), while the UV spectrum indicated that a conjugated aromatic ring was present in the molecule ($\lambda_{max}$ 226 nm). The $^1H$ NMR spectrum showed the signals for a *para*-substituted aromatic ring, two olefinic protons on a *trans* double bond,[$\delta_H$6.45 (*d*, $J$=16 Hz) and 7.71 (*d*, $J$=16 Hz)], two olefinic protons on an enol ether, and two hemiacetalic protons [$\delta_H$ 4.72 (*d*) and 5.29 (*d*)]. The $^{13}C$ NMR spectrum showed six and ten signals for a $\beta$-glucopyranose and a *p*-methoxycinnamoyl moiety, respectively (Table 1). The remaining nine signals consisted of one for a dihydropyran ring and one triplet ($\delta_C$ 69.2), two doublets ($\delta_C$78.9 and 79.0), and one singlet ($\delta_C$ 81.6) for carbons with hydroxyl substituents. These findings led to the conclusion that compound **1** was an iridoid glucoside *trans-p*-methoxycinnamoyl ester. A related compound, globularinin (**7**), has been isolated from *Globularia alypum* and has a cinnamoyl unit as the ester portion [2]. On alkaline hydrolysis of **1**, 10-descinnamoyl globularinin (**7**) was obtained [2], and the C-10 position of the ester substituent was confirmed by comparison of the $^{13}C$ NMR data for **1**, and **7** (see $\Delta\ \delta$ in Table 1). Therefore, the structure of compound **1** was elucidated to be 4″-methoxy-*E*-globularinin.

Compound **2** was found to have the same elemental composition as **1**. Other spectroscopic data were similar to those of **1**, except for the coupling constants of the olefinic protons, $\delta_H$ 5.94 (*d*, $J$=13 Hz) and 6.92 (*d*, $J$=13 Hz). Therefore, the structure of **2** was elucidated to be the *cis*-isomer of the *p*-methoxycinnamoyl moiety of **1**, namely 4″-methoxy-*Z*-globularinin.

Compound **3** was found to have an elemental composition (HR–FAB–MS) 14 mass units less than those of **1** and **2**. The $^1H$ and $^{13}C$ NMR spectra were similar to those of **1**, apart from the absence of the methoxyl signal found in the spectra as **1**. Therefore, structure of **3** was concluded to be 4″-hydroxy-*E*-globularinin.

Compounds **4** and **5** were found to have the same

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Table 1. $^{13}C$ NMR data for compounds **1–8** (100 MHz, $CD_3OD$)

| C | 1 | 2 | 6[a,b] | 7 | 3 | 4 | 5 | 8[a,b] |
|---|---|---|---|---|---|---|---|---|
| 1 | 96.4 | 96.4 | 96.3 | 95.9 | 96.4 | 93.8 | 93.8 | 93.5 |
| 3 | 141.7 | 141.7 | 141.6 | 141.8 | 141.7 | 140.8 | 140.7 | 140.5 |
| 4 | 106.6 | 106.5 | 106.4 | 106.0 | 106.6 | 106.0 | 106.1 | 105.8 |
| 5 | 39.1 | 39.0 | 38.9 | 38.9 | 39.1 | 38.9 | 38.9 | 38.4 |
| 6 | 78.9 | 79.0 | 78.6 | 78.8 | 79.0 | 84.3 | 84.3 | 83.8 |
| 7 | 79.0 | 79.2 | 78.6 | 80.1 | 79.0 | 85.8 | 85.8 | 85.4 |
| 8 | 81.6(−0.6)[c] | 81.4 | 81.4 | 82.2 | 81.7 | 80.6 | 80.5 | 80.2 |
| 9 | 45.0 | 45.2 | 44.6 | 44.1 | 45.0 | 49.4 | 49.3 | 48.9 |
| 10 | 69.2(+2.3)[c] | 69.2 | 69.0 | 66.9 | 69.1 | 66.6 | 66.3 | 66.4 |
| 1′ | 101.0 | 100.8 | 100.9 | 100.8 | 101.1 | 100.1 | 100.1 | 99.7 |
| 2′ | 74.9 | 74.8 | 74.4 | 74.8 | 74.8 | 74.8 | 74.9 | 74.4 |
| 3′ | 78.3 | 78.3 | 77.8 | 78.3 | 77.9 | 78.1 | 78.2 | 77.6 |
| 4′ | 71.2 | 71.4 | 70.8 | 71.4 | 71.2 | 71.5 | 71.6 | 71.1 |
| 5′ | 77.9 | 78.0 | 77.4 | 78.0 | 78.3 | 78.0 | 78.0 | 77.6 |
| 6′ | 62.5 | 62.6 | 62.2 | 62.6 | 62.5 | 62.9 | 62.9 | 62.5 |
| 1″ | 128.4 | 128.8 | | | 127.2 | 128.5 | 128.8 | |
| 2″ | 131.1 | 133.6 | | | 131.3 | 131.0 | 133.5 | |
| 3″ | 115.5 | 114.5 | | | 116.9 | 115.5 | 114.5 | |
| 4″ | 163.2 | 162.2 | | | 161.4 | 163.2 | 162.1 | |
| 5″ | 115.5 | 114.5 | | | 116.9 | 115.5 | 114.5 | |
| 6″ | 131.1 | 133.6 | | | 131.3 | 131.0 | 133.5 | |
| 7″ | 146.5 | 144.9 | | | 146.9 | 146.2 | 144.6 | |
| 8″ | 116.2 | 117.7 | | | 115.2 | 116.4 | 117.8 | |
| 9″ | 169.6 | 168.5 | | | 169.9 | 169.4 | 168.2 | |
| −OMe | 55.9 | 55.8 | | | | 55.9 | 55.8 | |

[a]Data for acyl moiety are omitted. [b]Data taken from ref. [3]. [c]$\Delta\delta_{1-7}$.

elemental composition as **1** and **2**. Their NMR spectra indicated the presence of the same moieties and functional groups as those of **1** and **2**, respectively. However, the coupling constants between H-6 and H-7 ($J=6$ Hz) were different from those of **1** and **2** ($J=4$ Hz), and the $^{13}C$ NMR chemical shifts of the iridoid portion were significantly different from those of **1** and **2**. The $^{13}C$ data for the iridoid moities of **4** and **5** were almost identical with those reported for globularimin (**8**) from *G. alypum* [2] (Table 1). Therefore, **4** and **5** were elucidated to be 4″-methoxy-*E*- and 4″-methoxy-*Z*-globularimin, respectively.

## EXPERIMENTAL

### *General*

$^1H$ NMR and $^{13}C$ NMR: 400 MHz and 100 MHz, respectively, with TMS as an int. standard. All other instrumentation was the same as reported previously [1].

### *Plant material*

Leaves of *P. subscandens* were collected on Ishigaki Island, Okinawa, and identified by one (A.T.) of the authors. A voucher specimen was deposited in the Herbarium of the Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine (PS-92-Okinawa).

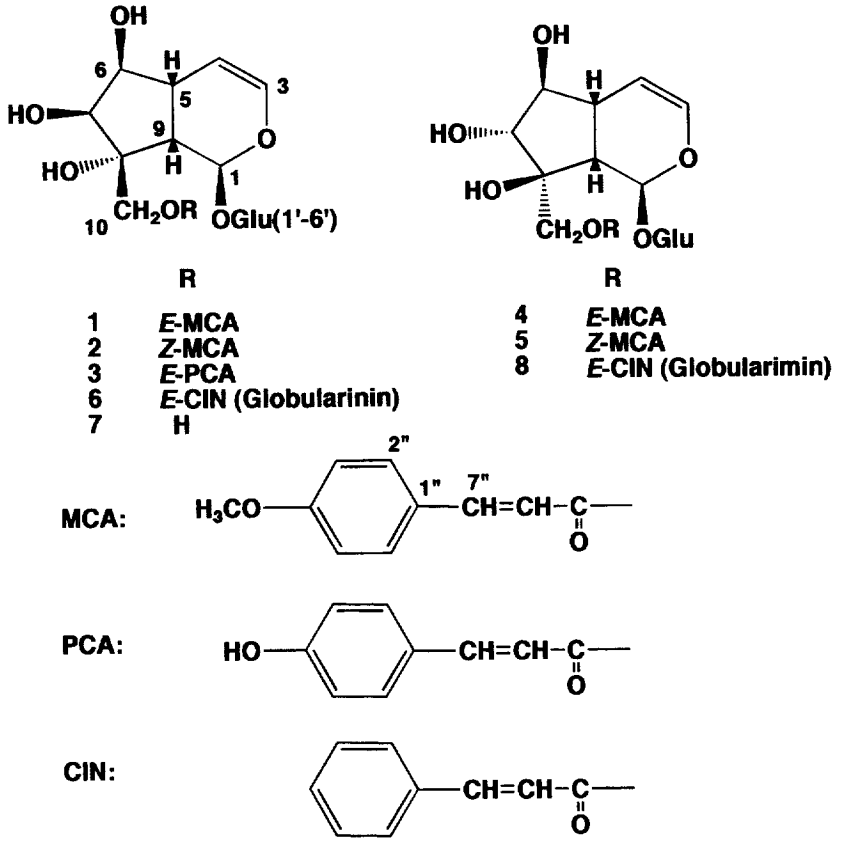
### *Extraction and purification*

Parts of the extraction and purification procedures were essentially the same as those reported previously [1].

The residue (1.85 g) of the 20%b MeOH eluate was subjected to RPCC (144 mg in fractions 80–92), DCCC (26 mg in fractions 27–31), and then HPLC ($H_2O$-MeOH 4:1) to give 13 mg of 3 (28 min).

The residue (9.38 g) of the 60%a MeOH eluate on Diaion HP-20 CC was subjected to silica gel (500 g) CC. $CHCl_3$ (11) and $CHCl_3$-MeOH mixtures [99:1 (21), 49:1 (21), 24:1 (41), 93:7 (41), 9:1 (41), 17:3 (41), 4:1 (41), and 3:1 (41)] were successively passed through the column and 500 ml-fractions were collected. The residue (609 mg in fractions 34–41) of the 10% MeOH eluate was separated successively by RPCC (109 mg in fractions 119–129), DCCC (41 mg in fractions 46–54), and then HPLC ($H_2O$-MeOH 13:7) to give 3 mg of **5** (37 min) and 22 mg of **4** (49 min).

The residue (10.0 g) of the 60%b MeOH eluate on Diaion HP-20 CC was subjected to silica gel (500 g) CC. $CHCl_3$ (1.51) and $CHCl_3$-MeOH mixtures [99:1 (21), 49:1 (21), 24:1 (41), 93:7 (41), 9:1 (61), 7:1 (61), 17:3 (61), 4:1 (61), and 3:1 (11)] were successively




passed through the column and 500 ml-fractions were collected. The residue (2.10 g in fractions 32–50) of the 12.5% MeOH eluate was subjected to RPCC (74 mg in fractions 121–132) and then DCCC. The residue (51 mg) of fractions 53–62 was purified by HPLC ($H_2O$-MeOH 3:2) to give 46 mg of **1** (26 min). The residue (12 mg) of fractions 47–52 on DCCC was also purified by HPLC ($H_2O$-MeOH 3:2) to give 7 mg of **2** (21 min).

*4″-Methoxy-Z-globularinin* (**1**)

Amorphous powder, $[\alpha]_D^{24}$ −74.8° (MeOH, *c* 1.04). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3325, 1680, 1625, 1600, 1505, 1420, 1250, 1170, 1070, 1015, 830; UV $\lambda_{max}^{MeOH}$ nm (log ε): 217 (3.97), 226 (4.01), 302sh (4.24), 308 (4.27); $^1$H NMR ($CD_3OD$): δ 2.39 (H, *dd*, *J*=6 and 11 Hz, H-9), 2.70 (H, *br tdd*, *J*=3, 8 and 11 Hz, H-5), 3.30 (H, *dd*, *J*=8 and 9 Hz, H-2′), 3.66 (H, *dd*, *J*=5 and 12 Hz, H-6′a), 3.82 (H, *dd*, *J*=2 and 12 Hz, H-6′b), 3.83 (3H, *s*, $-OCH_3$), 3.87 (H, *d*, *J*=4 Hz, H-7), 3.95 (H, *dd*, *J*=4 and 8 Hz, H-6), 4.34 (H, *d*, *J*=11 Hz, H-10a), 4.53 (H, *d*, *J*=11 Hz, H-10b), 4.72 (H, *d*, *J*=8 Hz, H-1′), 5.12 (H, *dd*, *J*=3 and 6 Hz, H-4), 5.29 (H, *d*, *J*=6 Hz, H-1), 6.32 (H, *dd*, *J*=2 and 6 Hz, H-3), 6.45 (H, *d*, *J*=16 Hz, H-8″), 6.96 (2H, *d*, *J*=9 Hz, $H_2$-3″ and 5″), 7.57 (2H, *d*, *J*=9 Hz, $H_2$-2″ and 6″), 7.71 (H, *d*, *J*=16 Hz, H-7″); $^{13}$C NMR ($CD_3OD$): Table 1: HR–FAB–MS (negative centroid) *m/z*:539.1764 ($C_{25}H_{31}O_{13}$ requires 539.1765).

*4″-Methoxy-Z-globularinin* (**2**)

Amorphous powder, $[\alpha]_D^{24}$ −51.5° (MeOH, *c* 0.45). UV $\lambda_{max}^{MeOH}$ nm (log ε): 223 (3.95), 301 (4.14)sh, 308 (4.16); $^1$H NMR ($CD_3OD$): δ 2.38 (H, *dd*, *J*=6 and 11 Hz, H-9), 2.67 (H, *br tdd*, *J*=3, 8 and 11 Hz, H-5), 3.27 (H, *dd*, *J*=8 and 9 Hz, H-2′), 3.66 (H, *dd*, *J*=5 and 12 Hz, H-6′a), 3.82 (3H, *s* $-OCH_3$), 3.83 (H, *d*, *J*=4 Hz, H-7), 3.83 (H, *dd*, *J*=2 and 12 Hz, H-6′ b), 3.94 (H, *dd*, *J*=4 and 8 Hz, H-6), 4.30 (H, *d*, *J*=11 Hz, H-10a), 4.44 (H, *d*, *J*=11 Hz, H-10b), 4.70 (H, *d*, *J*=8 Hz, H-1′), 5.10 (H, *dd*, *J*=3 and 6 Hz, H-4), 5.28 (H, *d*, *J*=6 Hz, H-1), 5.94 (H, *d*, *J*=13 Hz, H-8″), 6.31 (H, *dd*, *J*=2 and 6 Hz, H-3), 6.90 (2H, *d*, *J*=9 Hz, $H_2$-3″ and 5″), 6.92 (H, *d*, *J*=13 Hz, H-7″), 7.75 (2H, *d*, *J*=9 Hz, $H_2$-2″ and 6″); $^{13}$C NMR ($CD_3OD$): Table 1: HR–FAB–MS (negative centroid) *m/z*: 539.1771 ($C_{25}H_{31}O_{13}$ requires 539.1765).

*4″-Hydroxy-E-globularinin* (**3**)

Amorphous powder, $[\alpha]_D^{25}$ −76.2° (MeOH, *c* 0.81). UV $\lambda_{max}^{MeOH}$ nm (log ε): 211 (4.00), 228 (3.99), 311 (4.25); $^1$H NMR ($CD_3OD$): δ 2.39 (H, *dd*, *J*=6 and 10 Hz, H-9), 2.69 (H, *tdd*, *J*=3, 8 and 10 Hz, H-5), 3.27 (H,

*dd*, $J=8$ and 9 Hz, H-2′), 3.66 (H, *dd*, $J=5$ and 12 Hz, H-6′a), 3.82 (H, *dd*, $J=2$ and 12 Hz, H-6′b) 3.87 (H, *d*, $J=4$ Hz, H-7), 3.95 (H, *dd*, $J=4$ and 8 Hz, H-6), 4.32 (H, *d*, $J=12$ Hz, H-10a), 4.52 (H, *d*, $J=12$ Hz, H-10b), 4.71 (H, *d*, $J=8$ Hz, H-1′), 5.12 (H, *dd*, $J=3$ and 6 Hz, H-4), 5.29 (H, *d*, $J=6$ Hz, H-1), 6.32 (H, *dd*, $J=2$ and 6 Hz, H-3), 6.39 (H, *d*, $J=16$ Hz, H-8″), 6.81 (2H, *d*, $J=9$ Hz, $H_2$-3″ and 5″), 7.48 (2H, *d*, $J=9$ Hz, $H_2$-2″ and 6″), 7.68 (H, *d*, $J=16$ Hz, H-7″); $^{13}$C NMR ($CD_3OD$): Table 1; HR–FAB–MS (negative centroid) *m/z*: 525.1626 ($C_{24}H_{29}O_{13}$ requires 525.1608).

*4″-Methoxy-E-globularimin* (**4**)

Amorphous powder, $[\alpha]_D^{26}$ −90.4° (MeOH, *c* 1.38). IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3350, 2900, 1685, 1625, 1600, 1510, 1420, 1250, 1170, 1070–1010, 960, 830; UV $\lambda_{max}^{MeOH}$ nm (log ε):209 (4.00), 227 (4.03), 304 (4.26); $^1$H NMR ($CD_3OD$): δ 2.50 (H, *dd*, $J=5$ and 10 Hz, H-9), 2.68 (H, *dddd*, $J=2$, 4, 6 and 10 Hz, H-5), 3.21 (H, *dd*, $J=8$ and 9 Hz, H-2″), 3.67 (H, *dd*, $J=6$ and 12 Hz, H-6′a), 3.73 (H, *t*, $J=6$ Hz, H-6), 3.83 (3H, *s*, $-OCH_3$), 3.84 (H, *dd*, $J=2$ Hz and 12 Hz, H-6′b), 3.87 (H, *d*, $J=6$ Hz, H-7), 4.34 (H, *d*, $J=12$ Hz, H-10a), 4.53 (H, *d*, $J=12$ Hz, H-10b), 4.64 (H, *d*, $J=8$ Hz, H-1′), 5.10 (H, *dd*, $J=4$ and 6 Hz, H-4), 5.55 (H, *d*, $J=5$ Hz, H-1), 6.23 (H, *dd*, $J=2$ and 6 Hz, H-3), 6.41 (H, *d*, $J=16$ Hz, H-8″), 6.95 (2H, *d*, $J=9$ Hz, $H_2$-3″ and 5″), 7.56 (2H, *d*, $J=9$ Hz, $H_2$-2″ and 6″), 7.69 (H, *d*, $J=16$ Hz, H-7″); $^{13}$C NMR ($CD_3OD$): Table 1; HR–FAB–MS (negative centroid) *m/z*: 539.1756 ($C_{25}H_{31}O_{13}$ requires 539.1765).

*4″-Methoxy-Z-globularimin* (**5**).

Amorphous powder, $[\alpha]_D^{26}$ −96.0° (MeOH, *c* 0.17), UV $\lambda_{max}^{MeOH}$ nm (log ε): 206 (4.27), 224sh (4.11), 306 (4.15); $^1$H NMR ($CD_3OD$): δ 2.46 (H, *dd*, $J=6$ and 10 Hz, H-9), 2.66 (H, *dddd*, $J=2$, 4, 7 and 10 Hz, H-5), 3.21 (H, *dd*, $J=8$ and 9 Hz, H-2′), 3.68 (H, *dd*, $J=6$ and 7 Hz, H-6), 3.71 (H, *d*, $J=6$ Hz, H-7), 3.82 (3H, *s*, $-OCH_3$), 3.85 (H, *dd*, $J=2$ and 12 Hz, H-6b′), 4.30 (H, *d*, $J=12$ Hz, H-10a), 4.47 (H, *d*, $J=12$ Hz, H-10b), 4.62 (H, *d*, $J=8$ Hz, H-1′), 5.08 (H, *dd*, $J=4$ and 6 Hz, H-4), 5.51 (H, *d*, $J=6$ Hz, H-1), 5.90 (H, *d*, $J=13$ Hz, H-8″), 6.22 (H, *dd*, $J=2$ and 6 Hz, H-3), 6.88 (H, *d*, $J=13$ Hz, H-7″), 6.89 (2H, *d*, $J=9$ Hz, $H_2$-3″ and 5″), 7.73 (2H, *d*, $J=9$ Hz, $H_2$-2″ and 6″); $^{13}$C NMR ($CD_3OD$): Table 1; HR–FAB–MS (negative centroid) *m/z*:539.1763 ($C_{25}H_{31}O_{13}$ requires 539.1765).

*Alkaline hydrolysis of* **1** *to* **7**

4″-Methoxy-*E*-globularinin (**1**) (26 mg) was hydrolyzed with 10 ml of 0.1 M methanolic NaOH under an $N_2$ atmosphere for 30 min at 25°. The reaction mixture was neutralized by the addition of Amberlite IR-120 ($H^+$) and then filtered. The filtrate was taken to dryness under $N_2$ gas and then the residue was subjected to silica gel CC with increasing amounts of MeOH in $CHCl_3$ to give 14 mg (77%) of 10-descinnamoyl globularinin (**7**). Amorphous powder, $[\alpha]_D^{28}$ −70.5° (MeOH, *c* 1.12). UV $\lambda_{max}^{MeOH}$ (log ε): 206 (3.50); $^1$H NMR ($CD_3OD$): δ 2.40 (H, *dd*, $J=6$ and 10 Hz, H-9), 2.66 (H, *m*, H-5), 3.22 (H, *dd*, $J=8$ and 9 Hz, H-2′), 3.68 (H, *dd*, $J=5$ and 12 Hz, H-6′a a), 3.69 (H, *d*, $J=11$ Hz, H-10a), 3.72 (H, *d*, $J=11$ Hz, H-10b), 3.86 (H, *dd*, $J=2$ and 12 Hz, H-6′ b), 3.90 (H, *t*, $J=4$ Hz, H-6), 3.91 (H, *d*, $J=4$ Hz, H-7), 4.69 (H, *d*, $J=8$ Hz, H-1′), 5.04 (H, *dd*, $J=4$ and 6 Hz, H-4), 5.27 (H, *d*, $J=6$ Hz, H-1), 6.30 (H, *dd*, $J=2$ and 6 Hz, H-3); $^{13}$C NMR ($CD_3OD$): Table 1; HR–FAB–MS (negative centroid) *m/z*:379.1261 ($C_{15}H_{23}O_{11}$ requires 379.1240).

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