



## PREMNETHANOSIDES A AND B: PHENYLETHANOIDS FROM LEAVES OF *PREMNA SUBSCANDENS*

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**Key Word Index**—*Premna subscandens*; Verbenaceae; phenylethanoid; acteoside; verbascoside; premcoryoside; premnethanosides A and B.

**Abstract**—Two phenylethanoids, named premnethanosides A and B, were isolated from the leaves of *Premna subscandens*, along with known phenylethanoids, verbascoside (acteoside), decaffeoylacteoside and premcoryoside. The structures of the new compounds were elucidated by NMR spectroscopy and acetylation experiments. © 1997 Elsevier Science Ltd

### INTRODUCTION

Phenylethanoids are widely distributed in the Bignoniaceae, Labiatae, Scrophulariaceae, Verbenaceae and so on [1]. Many iridoid glucosides and phenylethanoids have been isolated from members of the *Premna* genus [2–5]. In this paper, we report on the isolation and structure elucidation of two new phenylethanoids from leaves of *P. subscandens* Merr.

### RESULTS AND DISCUSSION

Verbascoside (acteoside) (1), decaffeoylacteoside (2) and premcoryoside (3) were isolated as known compounds. Investigation of minor phenylethanoids from the plant afforded two new congeners (4 and 5).

Premnethanoside A (4) was assigned the molecular formula  $C_{40}H_{52}O_{12}$  by negative ion HR-FAB mass spectrometry. Its  $^{13}C$  NMR spectrum showed the presence of a feruloyl moiety with a *trans* double bond (Table 1), an additional aromatic ring having three protons in an ABX coupling system, hydroxyl and methoxyl ( $\delta_C$  147.6 and 147.4) groups and an ethanoid [ $\delta_C$  36.6 (*t*) and 72.2 (*t*)] moiety. These data were indicative that the main skeleton of 4 was that of martynoside (6) (Table 1). Further signals observed in the  $^{13}C$  NMR spectrum of 4 were due to two acetyl groups and a terminal  $\beta$ -glucopyranose. These substituents must be on the hydroxyl groups of the rhamnopyranose moiety, since the chemical shifts of the

rhamnopyranosyl carbons were different from those of 6. The presence of rhamnose in 4 was confirmed by GLC analysis of the methanolysis products. In the  $^1H$ - $^1H$  COSY spectrum, the H-2" signal appeared at  $\delta_H$  5.34 (*dd*,  $J = 2$  and 5 Hz) and showed cross peaks with the anomeric proton of the rhamnose [ $\delta_H$  5.22 (*d*,  $J = 2$  Hz)]. Another proton at  $\delta_H$  5.17 (*dd*,  $J = 5$  and 10 Hz) showed crossed peaks with the H-2" signal. These two protons were expected to be shifted downfield as a result of acetylation. Only the remaining hydroxyl group at the 4"-position must be linked by the terminal  $\beta$ -glucopyranose ( $J = 8$  Hz). Therefore, the structure of premnethanoside A was elucidated as 4. This was further confirmed by EI-MS analysis of the octaacetate (4a), which gave rise to a  $GLC(OAc)_4Rha(OAc)_2$  oxonium ion peak at  $m/z$  561.

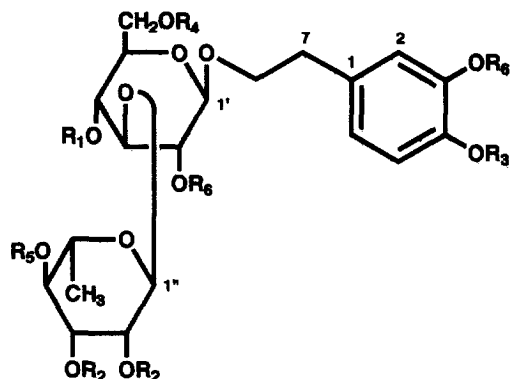
Premnethanoside B (5) had the same elemental composition as 4 and its spectroscopic data were similar to 4. The only significant difference observed was the coupling constants of the olefinic protons ( $J = 13$  Hz). Thus, premnethanoside B is the *cis* isomer of 4. We cannot rule out the possibility that the presence of the *cis* isomer is due to *trans* to *cis* isomerization in the plant itself and/or during the isolation procedures. However, the *trans* and *cis* isomers were stable during evaporation of the solvent from HPLC separation and the time taken to obtain the NMR measurements.

### EXPERIMENTAL

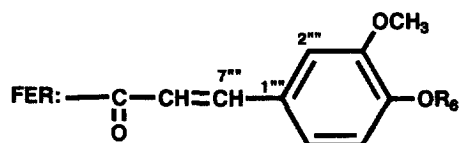
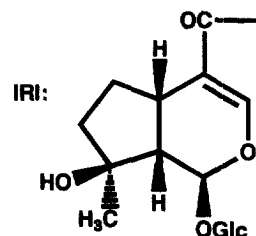
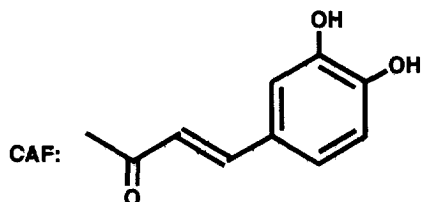
**General.**  $^1H$  and  $^{13}C$  NMR: 400 MHz and 100 MHz (TMS as int. standard), respectively; EI-MS: 70 eV.

**Plant material.** Leaves of *P. subscandens* were col-

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- 1  $R_1 = \text{CAF}, R_2 = R_3 = R_4 = R_5 = R_6 = \text{H}$
- 2  $R_1 = R_2 = R_3 = R_4 = R_5 = R_6 = \text{H}$
- 3  $R_1 = \text{CAF}, R_2 = R_3 = R_4 = R_5 = \text{H}, R_6 = \text{IRI}$
- 4  $R_1 = E\text{-FER}, R_2 = \text{Ac}, R_3 = \text{CH}_3, R_4 = R_5 = \text{H}, R_6 = \text{Glc}$
- 4a  $R_1 = E\text{-FER}, R_2 = R_4 = R_5 = \text{Ac}, R_3 = \text{CH}_3, R_6 = \text{Glc}(\text{OAc})_4$
- 5  $R_1 = Z\text{-FER}, R_2 = \text{Ac}, R_3 = \text{CH}_3, R_4 = R_5 = \text{H}, R_6 = \text{Glc}$
- 6  $R_1 = E\text{-FER}, R_3 = \text{CH}_3, R_2 = R_4 = R_5 = R_6 = \text{H}$



lected in August, 1992 in Ishigaki Islands, Okinawa. The plant was identified by one of the authors (A.T.) and a voucher specimen was deposited in the Herbarium of Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine (PS-92-Okinawa).

**Extraction and isolation.** The air-dried leaves of *P. subscandens* (840 g) was extracted and partitioned according to a previous experiment [5] to give a *n*-BuOH-soluble extract (56.3 g). The extract was subjected to cc on the highly porous synthetic resin, Diaion HP-20 (Mitsubishikasei Co. Ltd, Tokyo, H<sub>2</sub>O–MeOH), silica gel (Merck, CHCl<sub>3</sub> → CHCl<sub>3</sub>–MeOH) and ODS (Cosmosil, H<sub>2</sub>O–MeOH) and then DCCC (CHCl<sub>3</sub>–MeOH–H<sub>2</sub>O–*n*-PrOH: 9:12:8:2) and/or prep. HPLC (Inertsil, H<sub>2</sub>O–MeOH) to give **1** (1.26 g), **2** (32 mg), **3** (446 mg), **4** (10.1 mg) and **5** (3.3 mg).

**Known compounds isolated.** Verbascoside (**1**),  $[\alpha]_D^{23} - 81.9^\circ$  (MeOH, *c* 1.67) [6]; decaffeoylacteoside (**2**),  $[\alpha]_D^{25} - 41.1^\circ$  (MeOH, *c* 2.14) [4]; premcoryoside (**3**),  $[\alpha]_D^{25} - 56.5^\circ$  (MeOH, *c* 1.47) [5].

**Premnethanoside A (4).** Amorphous powder,  $[\alpha]_D^{28} - 78.7^\circ$  (MeOH, *c* 0.67). UV  $\lambda_{\text{max}}^{\text{MeOH}}$  nm (log  $\epsilon$ ): 207 (4.30), 219 (4.24), 230 (4.17) sh, 289 (4.06), 325 (4.20); <sup>1</sup>H NMR and <sup>13</sup>C NMR (CD<sub>3</sub>OD): Table 1; HR-FAB-MS (negative centroid) *m/z*: 897.2998 [M–H]<sup>–</sup> (C<sub>41</sub>H<sub>53</sub>O<sub>22</sub> requires 897.3028).

**Premnethanoside B (5).** Amorphous powder. UV  $\lambda_{\text{max}}^{\text{MeOH}}$  nm (log  $\epsilon$ ): 206 (4.34), 230 (4.15) sh, 289 (3.95), 321 (4.04); <sup>1</sup>H NMR (CD<sub>3</sub>OD):  $\delta$  1.24 (3H, *d*, *J* = 6 Hz, H<sub>3</sub>–6''), 1.98 and 2.08 (3H each, each *s*, –COCH<sub>3</sub> × 2), 2.81 (2H, *br t*, *J* = 7 Hz, H<sub>2</sub>–7), 2.96 (H, *t*, *J* = 8 Hz, H–2''), 3.43 (H, *dd*, *J* = 8 and 9 Hz, H–2'), 3.81 (3H, *s*, –OCH<sub>3</sub> at C–4), 3.89 (3H, *s*, –OCH<sub>3</sub>

at C–3'''), 4.05 (H, *ddd*, *J* = 7, 8 and 10 Hz, H–8b), 4.34 and 4.32 (H each, each *d*, each *J* = 8 Hz, H–1' and –1''), 4.94 (H, *t*, *J* = 9 Hz, H–4'), 5.15 (H, *d*, *J* = 2 Hz, H–1''), 5.18 (H, *dd*, *J* = 3 and 10 Hz, H–3''), 5.35 (H, *dd*, *J* = 2 and 3 Hz, H–2''), 5.78 (H, *d*, *J* = 13 Hz, H–8'''), 6.69 (H, *dd*, *J* = 2 and 8 Hz, H–6), 6.75 (H, *d*, *J* = 2 Hz, H–2), 6.80 (H, *d*, *J* = 8 Hz, H–5'''), 6.83 (H, *d*, *J* = 8 Hz, H–5), 6.94 (H, *d*, *J* = 13 Hz, H–7'''), 7.16 (H, *dd*, *J* = 2 and 8 Hz, H–6'''), 7.89 (H, *d*, *J* = 2 Hz, H–2'''); <sup>13</sup>C NMR (CD<sub>3</sub>OD): Table 1; HR-FAB-MS (negative centroid) *m/z*: 897.2997 [M–H]<sup>–</sup> (C<sub>41</sub>H<sub>53</sub>O<sub>22</sub> requires 897.3028).

**Acetylation of premnethanoside A (4).** About 2 mg of **4** was acetylated with 50  $\mu$ l each of Ac<sub>2</sub>O and pyridine at 25° for 18 hr. The reagents were removed under a stream of N<sub>2</sub> and dried *in vacuo* to give the octaacetate **4a**: <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  1.98 (3H, *s*), 1.99 (3H, *s*), 2.00 (9H, *s*), 2.05 (3H, *s*), 2.09 (3H, *s*), 2.10 (3H, *s*) (CH<sub>3</sub>CO– on alcoholic OH × 8), 2.30 (3H, *s*), 2.32 (3H, *s*) (CH<sub>3</sub>CO– on phenolic OH × 2); FAB-MS (negative centroid) (*m*-nitrobenzyl alcohol) *m/z*: 1233 [M–H]<sup>–</sup>; EI-MS (mass range: 300–1250) *m/z* (rel. int.): 1192 [M–CH<sub>2</sub>–C–O]<sup>+</sup> (2.3), 1150 [M–CH<sub>2</sub>–C–O × 2]<sup>+</sup> (0.7), 674 [M–Glc(OAc)<sub>4</sub>Rha(OAc)<sub>2</sub> + H]<sup>+</sup> (47), 632 [M–GLc(OAc)<sub>4</sub>Rha(OAc)<sub>2</sub>–CH<sub>2</sub> = C = O + H]<sup>+</sup> (43), 590 [M–Glc(OAc)<sub>4</sub>Rha(OAc)<sub>2</sub>–CH<sub>2</sub> = C = O × 2 + H]<sup>+</sup> (11), 561 [Glc(OAc)<sub>4</sub>Rha(OAc)<sub>2</sub>oxonium ion]<sup>+</sup> (5), 423 [M–C<sub>11</sub>H<sub>13</sub>O<sub>4</sub> (aglycone)–Glc(OAc)<sub>4</sub>Rha(OAc)<sub>2</sub>–CH<sub>2</sub> = C = O + H]<sup>+</sup> (99), 331 [Glc(OAc)<sub>4</sub>oxonium ion]<sup>+</sup> (100).

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Table 1.  $^{13}\text{C}$  and  $^1\text{H}$  NMR data for premnethanosides A (4) and B (5), and martynoside (6) ( $\text{CD}_3\text{OD}$ )\*

|                         | 4     |                               | 5     | 6†    |
|-------------------------|-------|-------------------------------|-------|-------|
|                         | C     | H                             | C     | C     |
| Aglycone                |       |                               |       |       |
| 1                       | 133.0 | —                             | 133.0 | 132.9 |
| 2                       | 113.0 | 6.73 ( <i>d</i> , 2)          | 112.9 | 113.0 |
| 3                       | 147.6 | —                             | 147.6 | 147.5 |
| 4                       | 147.4 | —                             | 147.4 | 147.4 |
| 5                       | 117.1 | 6.83 ( <i>d</i> , 8)          | 117.1 | 117.1 |
| 6                       | 121.2 | 6.87 ( <i>dd</i> , 2, 8)      | 121.2 | 121.1 |
| 7                       | 36.6  | 2.82 ( <i>br t</i> , 7)       | 36.6  | 36.5  |
| 8                       | 72.2  | 3.72 ( <i>ddd</i> , 6, 7, 10) | 72.2  | 72.1  |
|                         |       | 4.05 ( <i>ddd</i> , 7, 8, 10) |       |       |
| $\text{CH}_3\text{O}-$  | 56.6  | 3.81 ( <i>s</i> )             | 56.6  | 56.6  |
| Inner glucopyranose     |       |                               |       |       |
| 1'                      | 104.2 | 4.36 ( <i>d</i> , 8)          | 104.2 | 104.2 |
| 2'                      | 76.0  | 3.46 ( <i>dd</i> , 8, 9)      | 76.0  | 76.0  |
| 3'                      | 81.8  | 3.84 ( <i>t</i> , 9)          | 82.5  | 81.5  |
| 4'                      | 70.5  | 5.04 ( <i>t</i> , 9)          | 70.3  | 70.7  |
| 5'                      | 76.1  | 3.55‡                         | 76.0  | 76.2  |
| 6'                      | 62.4  | 3.65 ( <i>dd</i> , 6, 12)     | 62.4  | 62.4  |
|                         |       | 3.81‡                         |       |       |
| Rhamnopyranose          |       |                               |       |       |
| 1''                     | 100.1 | 5.22 ( <i>d</i> , 2)          | 100.4 | 102.9 |
| 2''                     | 73.2  | 5.34 ( <i>dd</i> , 2, 5)      | 73.2  | 72.4  |
| 3''                     | 71.8  | 5.17 ( <i>dd</i> , 5, 10)     | 71.7  | 72.1  |
| 4''                     | 77.8  | 3.65 ( <i>t</i> , 10)         | 77.8  | 73.8  |
| 5''                     | 68.9  | 3.81‡                         | 69.1  | 70.4  |
| 6''                     | 18.8  | 1.21 ( <i>d</i> , 6)          | 18.6  | 18.5  |
| Outer glucopyranose     |       |                               |       |       |
| 1'''                    | 105.0 | 4.32 ( <i>d</i> , 8)          | 105.2 |       |
| 2'''                    | 74.9  | 2.92 ( <i>dd</i> , 8, 9)      | 75.0  |       |
| 3'''                    | 78.2  | 3.25 ( <i>t</i> , 9)          | 78.4  |       |
| 4'''                    | 71.5  | 3.12 ( <i>dd</i> , 9, 10)     | 71.6  |       |
| 5'''                    | 77.9  | 3.18 ( <i>ddd</i> , 2, 6, 10) | 78.0  |       |
| 6'''                    | 63.5  | 3.55 ( <i>dd</i> , 6, 12)     | 63.0  |       |
|                         |       | 3.81‡                         |       |       |
| Feruloyl moiety         |       |                               |       |       |
| 1'''                    | 127.6 | —                             | 127.9 | 127.7 |
| 2'''                    | 111.9 | 7.19 ( <i>d</i> , 2)          | 112.9 | 111.9 |
| 3'''                    | 151.1 | —                             | 150.0 | 150.8 |
| 4'''                    | 149.5 | —                             | 148.4 | 149.4 |
| 5'''                    | 116.7 | 6.83 ( <i>d</i> , 8)          | 115.6 | 116.5 |
| 6'''                    | 124.5 | 7.09 ( <i>dd</i> , 2, 8)      | 117.1 | 124.3 |
| 7'''                    | 148.2 | 7.66 ( <i>d</i> , 16)         | 147.8 | 147.8 |
| 8'''                    | 114.9 | 6.36 ( <i>d</i> , 16)         | 115.5 | 115.1 |
| 9'''                    | 168.3 | —                             | 167.0 | 168.3 |
| $\text{CH}_3\text{O}-$  | 56.6  | 3.90 ( <i>s</i> )             | 56.7  | 56.6  |
| $\text{CH}_3\text{CO}-$ | 20.7  | 1.99( <i>s</i> )              | 20.8  |       |
|                         | 21.1  | 2.07 ( <i>s</i> )             | 21.1  |       |
| $\text{CH}_3\text{CO}-$ | 171.7 | —                             | 171.7 |       |
|                         | 172.2 | —                             | 172.3 |       |

\* Letters and numbers in parentheses are multiplicities and coupling constants in Hz.

† Data taken from ref. [6].

‡ These signals could not be assigned due to overlapping of many signals.

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