Three New Sesquiterpenoid Glucosides of Ficus pumila Fruit

Junichi Kitajima,* Kaoru Kimizuka, and Yasuko Tanaka


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As the glycosyl constituents of Ficus pumila L. fruits (Moraceae, ñhitabì in Japanese) has been used in Chinese folk medicine as antitumor, antiinflammatory and tonic medicament.1

In previous papers,2) we reported on the sterol and triterpenoid components of this fruit. In this paper, we describe the isolation and characterization of three new sesquiterpenoid glucosides from the fruit, together with identification of the known glycosides.

The methanolic extract of the fresh fruit was suspended in water and then extracted with ether, ethyl acetate and n-butanol, successively. The n-butanol extract was treated as described in Experimental to isolate three new sesquiterpenoid glucosides, pumilaside A (1), pumilaside B (2) and pumilaside C (3), together with the known glycosides 4–6, which were identified as benzyl β-D-glucopyranoside,3) (E)-2-methyl-2-butenyl β-D-glucopyranoside,4) and rutin5) by comparison of 1H- and 13C-NMR spectra with those of authentic samples.

Pumilaside A (1), C31H38O8, amorphous powder, [α]D 23° (+28°) showed the [M+K]+, [M+Na]+, [M–C6H12O6]+ ion peaks at m/z 457, 441, 419 and 239 on the positive FAB-MS. The 1H, 13C- and 13C–1H correlation spectroscopy (COSY) NMR spectral data (Tables 1 and 2) showed the presence of one β-glucopyranosyl, two tert-methyls, four sec-methylene, four methylenes (one of them oxygenated), three quaternary carbons (one of them oxygenated). From the HMBC correlation data: H-5/C-1, C-3, C-4, C-6, C-9, C-10, C-11, C-12 and C-14; H-6/C-7, C-9, C-11, C-12 and C-14; H-7/C-5, a partial structure as described in Fig. 1 was obtained. Then, 1 was suggested to be a glucoside of eudesmane-type sesquiterpene having two hydroxyl groups at C-1 and C-4. The position of the glycosyl unit was ascertained to be C-1 in the same way as described for 1. As the NOE interactions between the signals of H-14/Hax.-2, H-6, H-7, Heq.-9 and H-15; H-15/Heq.-3 and H-6 were observed in the NOESY correlation data: H-1/H2-2; H-6/H5-5 and H-7/H7-8 (δ 1.63) and H-11, a partial structure as described in Fig. 1 was obtained. Then, 1 was suggested to be a glucoside of eudesmane-type sesquiterpenoid having two hydroxyl groups at C-1 and C-4. The position of the glycosyl unit was ascertained to be C-1 in the same way as described for 1. As the NOE interactions between the signals of H-14/Hax.-2, H-6, H-7, Heq.-9 and H-15; H-15/Heq.-3 and H-6 were observed in the NOESY spectrum of 2 (Fig. 2), the orientation of H-6, H-7, H-14 and H-15 was concluded to be the same as 1. Moreover, NOE interactions between H-5/H-1, H-12 and H-13 (Fig. 2), and the small coupling constant (4.5 Hz) between Hax.-6/H-7 suggested that the orientation of H-1, H-5 and the isopropyl group should be axial in the opposite direction to H-6, H-14 and H-15. So, 1 could be assumed to be a 6-O-β-D-glucopyranosyl of 1α,4β,6β-trihydroxyeudesmane or its enantiomer. Enzymatic hydrolysis of 1 gave an aglycone (1a, C15H23O3, amorphous powder, [α]D 24° (+6°) and D-glucose, and the absolute configuration at C-6 of 1 was indicated as R by the values of the glycosylation shift of the α- and the β-pro-S-side-carbons, and the chemical shift of the glucosyl anomeric carbon as shown in Table 3.6) Thus, 1 was characterized as (1S,4S,5R,6R,7S,10S)-1,4,6-trihydroxyeudesmane 6-O-β-D-glucopyranoside as described in Fig. 2.

Pumilaside B (2), C21H38O10, white powder [mp 195–197°C (dec.)], [α]D 24° (−19°) showed the [M+K]+, [M+Na]+, [M–C6H12O6]+ and [M–C6H12O6–H2O+H]+ ion peaks at m/z 439, 423, 221 and 203 in the positive FAB-MS. The 1H, 13C- and 13C–1H COSY NMR spectral data of 2 (Tables 1 and 2) showed the presence of one β-glucopyranosyl, four tert-methyls, four methylenes, four methines (one of them oxygenated), three quaternary carbons (one of them oxygenated). From the HMBC correlation data: H-5/C-1, C-3, C-4, C-6, C-9, C-10, C-11, C-14 and C-15; H-6/C-4, C-7, C-11, C-12 and C-13; H-7/C-5, C-6, C-9, C-11, C-12 and C-13; H-9/C-12, C-7, C-11 and C-13; H-12/C-6, C-7, C-11 and C-13; H-13/C-6, C-7, C-11 and C-12; H-14/C-1, C-5, C-9 and C-10; H-15/C-3, C-4 and C-5, and 1H–1H COSY correlation data: H-1/H2-2; H-6/H5-5 and H-7/H7-8 (δ 1.63) and H-11, a partial structure as described in Fig. 1 was obtained. Then, 2 was suggested to be a glucoside of maaliane-type sesquiterpenoid having two hydroxyl groups at C-1 and C-4. The position of the glycosyl unit was ascertained to be C-1 in the same way as described for 1. As the NOE interactions between the signals of H-14/Hax.-2, H-6, H-7, Heq.-9 and H-15; H-15/Heq.-3 and H-6 were observed in the NOESY spectrum of 2 (Fig. 2), the orientation of H-6, H-7, H-14 and H-15 was concluded to be the same as 1. Moreover, NOE interactions between H-5/H-1, Hax.-3 and H-12 were observed in its NOESY spectrum (Fig. 2), and the orientation of H-1 and H-5 was opposite to H-6, H-7, H-14 and H-15. So, 2 was considered to be 1-O-β-glucopyranosyl of 1α,4β-dihydroxymaaliane or its enantiomer. Enzymatic hydrolysis of 2 gave an aglycone (2a, C15H23O3, mp 172–175°C, [α]D 24° (+10°) and D-glucose, and the values of the glycosylation shift of the α- and the β-pro-S-side-carbons, and the chemical shift of the glucosyl anomeric carbon (Table 3) suggested the absolute configuration at C-1 of 2 was S.6) From these facts, 2 was determined as (1S,4S,5S,6R,7R,10S)-

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Table 1. 1H-NMR Spectral Data for 1, 2 and 3

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
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</thead>
<tbody>
<tr>
<td>H-1</td>
<td>3.69 (1H, J=7.0 Hz)</td>
<td>3.58 (1H, J=7.0 Hz)</td>
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<tr>
<td>H2-2</td>
<td>1.90 (2H, m)</td>
<td>1.98 (1H, br ddd, J=13.0, 7.0, 3.0 Hz, H-α)</td>
</tr>
<tr>
<td>H3-3</td>
<td>1.93 (2H, m)</td>
<td>2.42 (1H, ddd, J=13.0, 12.0, 7.0, 3.0 Hz, H-β)</td>
</tr>
</tbody>
</table>

Solvent: pyridine-d_5 (500 MHz). δ in ppm from TMS.

Table 2. 13C-NMR Spectral Data for 1, 2, 2a, 3 and 3a

<table>
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<tr>
<th>Glucoside and carbon</th>
<th>Δδ (δ glucosyl - δ aglycone) or δ</th>
</tr>
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<tr>
<td>1 α-Carbon (C-6)</td>
<td>19.66</td>
</tr>
<tr>
<td>1 α-Carbon (C-1)</td>
<td>8.09</td>
</tr>
<tr>
<td>1 β-pro-S-side-carbon (C-7)</td>
<td>6.10</td>
</tr>
<tr>
<td>1 β-pro-S-side-carbon (C-10)</td>
<td>0.16</td>
</tr>
</tbody>
</table>

Solvent: pyridine-d_5 (125 MHz). δ in ppm from TMS.

Table 3. Glycosylation Shift and Glucosyl C-1 Chemical Shift of 1 and 2

<table>
<thead>
<tr>
<th>Glucoside and carbon</th>
<th>Δδ (δ glucosyl - δ aglycone) or δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 α-Carbon (C-6)</td>
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<td>0.16</td>
</tr>
</tbody>
</table>

Solvent: pyridine-d_5 (125 MHz). δ in ppm from TMS.

1,4-dihydroxymallane 1-O-β-D-glucopyranoside as described in Fig. 2.

Pumilaside C (3, C_9H_16O_6S) amorphous powder, [α]_D^24 −17.0° showed the [M+K]^+, [M+Na]^+ and [M–C_2H_5O]+ ions at m/z 439, 423 and 221 in the positive FAB-MS. The ^1H and ^13C-H COSY NMR spectral data of 3 (Tables 1 and 2) showed the presence of one β-glucopyranosyl, four tert-methyls, four methylenes, three methines, two oxygenated quaternary carbons and one trisubstituted double bond. The results of HMBC correlation: H-1/C-3, C-6, C-10 and C-14; H-5/C-1, C-3, C-6 and C-15; H-9 (δ
from plants of the *Ficus* genus.

**Experimental**

HPLC separation was carried out on a JASCO chromatography (980-sys-tem) with a JASCO 930 RI detector, JASCO OR-970 chiral detector and ODS-3251-D [Senshupak, column size, 8x250 mm, Symmetry Prep C18 [Waters, column size, 7.8x300 mm], Megapak SIL C18-10 [JASCO, column size, 7.5x250 mm], carbohydrate analysis [Waters, column size, 3.9x300 mm]] the other instruments used and the experimental conditions for the spectral data and for chromatography were the same as in the preceding paper.261

**Extraction and Separation of 1 to 6**  
*F. pumila* L. was collected at Gushikawa City, Okinawa Prefecture, Japan, in March 1994. The fresh fruit (28 kg) was extracted with methanol (32 l) at room temperature. After evaporation of the solvent, the residue (987 g) was suspended with water and successively extracted with ether, ethyl acetate and n-butanol. Removal of the solvent from each phase gave an ether (43.1 g), ethyl acetate (6.5 g), and n-butanol (35.1 g) and an aqueous (889 g) residue. The n-butanol residue was subjected to column chromatography on Amberlite XAD-2 (H2O) to afford water eluate (14.4 g) and methanol eluate (20.6 g). The methanol eluate fraction was chromatographed on Sephadex LH-20 (MeOH) which furnished four fractions. Fraction 2 (5.7 g) was purified by silica gel [CHCl3–MeOH (9 : 1)] chromatography to afford six fractions. From the second fraction, 4 (2 mg) and 5 (3 mg) were isolated by Sephadex LH-20 (MeOH). Lobar RP-8 column (25% MeOH) and silica gel [CHCl3–MeOH (9 : 1)] chromatography and HPLC using Symmetry Prep C18 (20% MeOH) and ODS-3251-D (10% MeOH). From the third fraction (140 mg), C18 (13 mg) was isolated by Sephadex LH-20 (MeOH), Lobar RP-8 column (30% MeOH) silica gel [CHCl3–MeOH-H2O (4 : 1 : 0.1)] chromatography and HPLC using carbohydrate analysis column (95% CH3CN). From the fourth fraction (50 mg), 2 (22 mg) was isolated by Sephadex LH-20 (MeOH), silica gel [CHCl3–MeOH-H2O (4 : 1 : 0.1)] chromatography and HPLC using Megapak SIL C18 (95% CH3CN). From the fifth fraction (35 mg), 3 (11 mg) was isolated by Sephadex LH-20 (MeOH), silica gel [CHCl3–MeOH-H2O (4 : 1 : 0.1)] chromatography and HPLC using Megapak SIL C18 (95% CH3CN). Fraction 3 (3.2 g) was purified by Sephadex LH-20 (MeOH) and Lobar RP-8 column (40% MeOH) to get 6 (35 mg).

The following compounds were identified by comparison with authentic compounds.

Benzy1 β-d-glucopyranoside (4), (E)-2-methyl-2-butenyl β-d-glucopyra-noside (5) and rutin (6).


**Enzymatic Hydrolysis of 1, 2 and 3**  
Glycoside 1 (4 mg), 2 (6 mg) and 3 (3 mg) were each dissolved in water (5 ml) with hespiridinase (3 mg), and the sugar fractions. The sugar fractions were passed through Sephadex LH-20 to give syrups. They were analyzed by HPLC [column, carbohydrate analysis (Waters: size, 3.9x250 mm), Symmetry Prep C18 (20% MeOH)] for C21H38NaO8: 441.2465, 419.2663 [M+H]+ (Calcd for C21H38NaO8: 441.2465), 399 [M–H2O+H]+ (base).

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References