Structures and Hypotensive Effect of Flavonoid Glycosides in Unshiu Peeling. II†.

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We describe the structures and hypotensive effects of three additional glycosides (**1**, **2** and **3**) obtained through further investigation on unshiu peelings. The structures 3-hydroxy-3', 4', 5, 6, 7, 8-hexamethoxyflavone 3- β -D-glucoside (**1**), limocitrin 3-O-[5- α -glucosyl-3-hydroxy-3-methylglutaryl (1 $\rightarrow$ 2)]- β -glucoside (**2**) and narirutin-4'-glucoside (**3**) were established by UV, ^1H -NMR and ^{13}C -NMR spectroscopy, and chemical evidence. Compounds **1** and **3** showed strong depressive effects, when intravenously injected with stroke-prone spontaneously hypotensive rats (0.5~1.0 mg dose/100 g body weight). Among these compounds, **1** was a new flavonoid glycoside, and **2** and **3** were found for the first time in unshiu.

In the previous paper, we reported six flavonoid glycosides, limocitrin 3- α -L-rhamnoside, apigenin 3, 6-di-C-glucoside, narirutin, rutin and narcissin isolated from *Citrus unshiu* peelings and their depressive effect on blood pressure¹⁾. We wish to describe herein the structures and hypotensive effects of three additional glycosides (**1**, **2** and **3**) which have been obtained by further investigation on unshiu peelings.

Thus, these glycosides were isolated by repeated silica gel column chromatography of the crude flavonoid fractions which had been prepared¹⁾ by gel filtration. The molecular weight of **1** was found to be 580 by FAB-MS which showed characteristic peaks at m/z 419 [aglycone+H]⁺, m/z 581 [M+H]⁺ and m/z 603 [M+Na]⁺. Maximum absorptions at 254 and 343 nm were shown in its UV spectrum in ethanol. These absorptions did not shift significantly upon addition of sodium acetate or aluminum chloride. These facts suggest that **1** did not have functional groups such as 7-hydroxy-, 4'-hydroxy-4-oxo-, 3-hydroxy-4-oxo- and *o*-dihydroxyl-systems³⁾. The ^1H -NMR spectrum of **1** indicates the presence of aromatic signals of an

ABX type at δ 7.87 ($J=2$ Hz), 6.96 ($J=9$ Hz) and 7.85 ($J=2$ and 9 Hz), respectively, assignable to H_{2'}, H_{5'} and H_{8'}, six methoxy groups at δ 3.89~4.08, and an anomeric proton of a β -glucoside moiety at δ 4.88 ($J=7$ Hz). The structure

Fig. 1 Structure of **1** and **1a**.

of a hexamethoxyflavonol glycoside is supported by the appearance of twenty-seven signals including six carbon signals of β -glucoside and six methoxy carbon signals in the ^{13}C -NMR spectrum. Acid hydrolysis of **1** gave D-glucose ($[\alpha]_D +42.2^\circ$, $c=0.1$, H_2O) and aglycone (**1a**). The ^1H -NMR spectrum of **1a** was similar to that of **1** except for the absence of signals due to the sugar protons of **1**. The UV spectrum of **1a** in ethanol shows maximum absorptions at 258 and 369 nm, which exhibit no shift on the addition of sodium acetate. However, the band I (369 nm) indicates a bathochromic shift of 67 nm when aluminum chloride was added, suggesting the presence of 3-hydroxy-4-oxo- system³⁾ in **1a**. Therefore, the structure of 3-hydroxy-3', 4', 5, 6, 7, 8-hexamethoxyflavone⁴⁾ was assigned to **1a** and **1** was determined as 3-hydroxy-3', 4', 5, 6, 7,

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Table-I Hypotensive effect.

Compound	Maximal decrease of blood pressure (mmHg) [Time after administration (h)]
1	-24 ^a [0.5]
2	-47 ^b [1]

^aDose : 0.5 mg/100 g body weight, i.v.^bDose : 1.0 mg/100 g body weight, i.v.

8-hexamethoxyflavone 3- β -D-glucoside.

Compound **2** was identified to be limocitrin 3-O-[[5- α -glucosyl-3-hydroxy-3-methylglutaryl (1 $\rightarrow$ 2)]- β -glucoside] by comparing its UV, FAB-MS and ¹H-NMR spectra with those of an authentic sample previously isolated from lemon peels⁵⁾.

The UV spectral data, specific rotation and melting point of **3** were identical with those of an authentic specimen of narirutin-4'-glucoside⁶⁾.

Depressive effect on blood pressure were examined for these flavonoid glycosides **1** and **3**, and the results are shown as average values of triplicates (**Table-I**). The blood pressure of stroke-prone spontaneously hypotensive rats (SHR-SP) showed the maximum decrease of 24 mmHg for **1** (0.5 mg dose/100 g body weight) at 30 min and 47 mmHg for **3** (1.0 mg dose/100 g body weight) at 1 h after the intravenous administration. Accordingly, flavonoid glycosides **1** and **3** from unshiu peelings are regarded as important constituents having depressive effect for SHR-SP.

Among these compounds isolated by the present study, **1** was found to be a new flavonoid glycoside, and also **2** and **3** were found for the first time in unshiu.

Experimental

Analytical instruments. The optical rotation was measured with a Japan Spectroscopic DIP-140. FAB-MS spectra were obtained with JEOL JMS-HX 100 and JMA-DA 5000 instruments under xenon bombardment (6.0 keV). UV spectra were taken with a Hitachi 323 visible-ultraviolet autoanalyzer. NMR spectra were measured with a JEOL-FX 200 in CDCl₃, CD₃OD or DMSO-*d*₆ (200 MHz for ¹H and 50 MHz for ¹³C).

Separation and purification. Croude fla-

vonoids were obtained from unshiu peelings (3100 g) by the conventional method¹⁾. Constituents **1**, **2** and **3** were isolated by repeated chromatography of the gel-filtered fractions No. 8, No. 13, and No. 9¹⁾, respectively in a column of silica gel with CHCl₃-MeOH (9 : 1) or CHCl₃-MeOH-AcOH-H₂O (70 : 15 : 12 : 3) as an eluant.

Acid hydrolysis of 1 and 3. A solution of 10 mg of a sample in 5 ml of 0.5 M-HCl was heated on a steam bath for 1 h. Cooling, dilution with water, and extraction with EtOAc gave an aglycone.

Measurement of the blood depressive effect. Each substance was intravenously injected [0.5 ~ 1.0 mg dose/100 g body weight (220 ~ 250 g)] in SHR-SP, whose blood pressure was measured chronologically using the tail pulse pick-up method without anesthesia.

3-Hydroxy-3', 4', 5, 6, 7, 8-hexamethoxyflavone 3- β -D-glucoside (1). Colorless needles (mp 111 ~ 112°C); FAB-MS : *m/z* 581 [M+H]⁺, 603 [M+Na]⁺, 419 [aglycone+H]⁺ (100 %); UV $\lambda_{\max}^{\text{EtOH}}$ nm : 254, 269 s, 343; $\lambda_{\max}^{\text{AcONa-EtOH}}$ nm : 256, 270 s, 345; $\lambda_{\max}^{\text{AlCl}_3\text{-EtOH}}$ nm : 256, 270 s, 343; ¹H-NMR $\delta_{\text{Me}_4\text{Si}}^{\text{CDCl}_3}$: 3.89 ppm (3 H, s, OMe), 3.90 (3 H, s, OMe), 3.92 (6 H, s, OMe $\times$ 2), 3.97 (3 H, s, OMe), 4.08 (3 H, s, OMe), 4.88 [1 H, d, *J*=7 Hz, β -Glc(H_{1''})], 6.96 (1 H, d, *J*=9 Hz, H_{5'}), 7.85 (1 H, d.d, *J*=2, 9 Hz, H_{6'}), 7.87 (1 H, d, *J*=2 Hz, H_{2'}); ¹³C-NMR $\delta_{\text{Me}_4\text{Si}}^{\text{CD}_3\text{OD}}$: 55.6 ppm (q, OMe), 60.7 (t, C_{6''}), 61.3 (q, OMe), 61.4 (q, OMe), 61.7 (q, OMe $\times$ 2), 61.8 (q, OMe), 69.8 (d, C_{4''}), 74.3 (d, C_{2''}), 76.5 (d, C_{3''}), 77.4 (d, C_{5''}), 100.6 (d, C_{1''}), 100.6 (s, C₁₀), 111.4 (d, C_{2'}), 112.5 (d, C_{5'}), 121.4 (d, C_{6'}), 122.8 (s, C_{1'}), 135.5 (s, C₈), 137.4 (s, C₃), 143.3 (s, C₉), 146.1 (s, C₆), 147.2 (s, C₇), 148.0 (s, C₅), 150.7 (s, C₂), 150.8 (s, C_{3'}), 153.3 (s, C_{4'}), 172.1 (s, C₄).

3-Hydroxy-3', 4', 5, 6, 7, 8-hexamethoxyflavone (1a). Colorless needles (mp 138 ~ 140°C; lit.⁴⁾ 141 ~ 143°C; UV $\lambda_{\max}^{\text{EtOH}}$ nm : 258, 369; $\lambda_{\max}^{\text{AcONa-EtOH}}$ nm : 258, 369; $\lambda_{\max}^{\text{AlCl}_3\text{-EtOH}}$ nm, 270, 434; ¹H-NMR $\delta_{\text{Me}_4\text{Si}}^{\text{CDCl}_3}$: 3.96 ppm (3 H, s, OMe), 3.97 (3 H, s, OMe), 3.98 (6 H, s, OMe $\times$ 2), 4.03 (3 H, s, OMe), 4.11 (3 H, s, OMe), 7.02 (1 H, d, *J*=8 Hz, H_{5'}), 7.90 (1 H, d, *J*=2 Hz, H_{2'}), 7.92 (1 H, d.d, *J*=2, 8 Hz, H_{6'}).

Narirutin-4'-glucoside (3). Pale yellow needles (mp 196~199°C; lit.⁹⁾ 189~200°C); FAB-MS: m/z 765 $[M+Na]^+$, 787 $[M-H+2Na]^+$; UV $\lambda_{\max}^{\text{EtOH}}$ nm: 285, 323; $\lambda_{\max}^{\text{AcONa-EtOH}}$ nm: 285, 325; $\lambda_{\max}^{\text{AlCl}_3\text{-EtOH}}$ nm: 309, 342, 386; $^1\text{H-NMR}$ $\delta_{\text{Me}_2\text{Si}}^{\text{DMSO}-d_6}$: 1.10 ppm (3H, d, $J=6$ Hz, rhamnose-Me), 2.86 (1H, d, $J=3$, 17 Hz, H_3), 4.54 (1H, d, $J=1$ Hz, α -rhamnose ($H_{1''}$)), 5.58 (1H, d, $J=3$, 12 Hz, H_2), 6.14 (2H, each s, H_6 , H_8), 7.08 (2H, d, $J=8$ Hz, $H_{3',5'}$), 7.46 (2H, d, $J=8$ Hz, $H_{2',6'}$), 11.70 (1H, s, C₅-OH); $[\alpha]_D: -61.1^\circ$ ($c=0.46$, in Pyridine).

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温州みかん果皮中のフラボノイド配糖体の構造及び血圧降下作用 (その 2)

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著者らは前報に引き続き温州みかん果皮中のフラボノイド配糖体について再検討し、新たに3種のフラボノイド配糖体を単離した。各成分の構造は各種スペクトルの測定結果から 3-ヒドロキシ-5,6,7,8,3',4'-ヘキサメトキシフラボン 3- β -D-グルコシド (1), リモシトリン 3-O-{[5- α -グルコシル-3-ヒドロキシ-3-メチルグルタリル(1 $\rightarrow$ 2)]- β -グルコシド} (2) 及びナリルチン-4'-グルコシド (3) と決定した。また 1 及び 3 の血圧降下作用について検討したところ、1 及び 3 に強い血圧降下作用が認められた。本研究で単離した成分のうち 1 は新規化合物であり、2 及び 3 は温州みかん果皮中から初めて見いだした成分である。