Note

Structure and Hypotensive Effect of Flavonoid Glycosides in Sudachi Peelings II†

Hiroyasu KUMAMOTO, Yoshiharu MATSUBARA, Yoshitomi IIZUKA,* Kozo OKAMOTO** and Katsumi YOKOI***

*Department of Applied Chemistry, Faculty of Science and Engineering, and *Research Institute of Food Science, Kinki University, Kowakae, Higashiosaka 577, Japan*
***Kinki University School of Medicine, Sayamacho, Minamikawachi 588, Japan*
****Japan Sangaria Beverage Co., Ltd., Nakano, Higashisumiyoshi-ku, Osaka 546, Japan*

Previously, we have reported the separation purification, structural determination and hypotensive effect of six flavonoid glycosides from green peel of *Citrus sudachi*.[1] In this paper, we report the isolation structural determination and hypotensive effect of a new flavone glycoside, 4′-β-D-glucosyl-sudachitin 7-0-(3-hydroxy-3-methylglutalate) (**1**).

The molecular weight of **1** was decided as 666 by FAB-MS by showing *m/z* 667 $[M+H]^+$ and *m/z* 689 $[M+Na]^+$. In the UV spectrum of **1**, the band II (284 nm) in EtOH did not undergo a large shift with the addition of sodium acetate. However, the band I (337 nm) indicated a bathochromic shift of 63 nm with the addition of aluminum chloride. These facts show that no hydroxyl groups existed at the C_7 position and that a hydroxyl group was located at the C_5 position.[2] ^{1}H-NMR data showed three aromatic protons of the $C_{2'}$, $C_{5'}$ and $C_{6'}$ positions, three methoxyl groups, an anomeric proton of β-glucose, and methyl and methylene protons of 3-hydroxy-3-methylglutalate. Characteristic data of the ^{13}C-NMR spectrum for **1** showed eighteen carbons of trimethylflavone, six carbons of glucose and six carbons of 3-hydroxy-3-methylglutaric acid monoester. After the alkaline hydrolysis of **1**, sudachiin A[1,3] was also identified on the basis of spectral evidence. On the other hand, the acidic hydrolysis of **1** gave sudachitin[4] and D-glucose ($[\alpha]_D$ +47.8°C, $c=0.1$, H_2O). Therefore, compound **1** was a new flavonoid having 3-hydroxy-3-methylglutalate and was determined as 4′-β-D-glucosylsudachitin 7-*O*-(3-hydroxy-3-methylglutalate). The depressive effect on blood pressure was examined for **1**, and the result is shown as the average value of three results. The blood pressure of SHR-SP decreased by 13 mmHg (1 mg/100 g of body weight) at 30 min after the intravenous administration.

Compound **1** isolated by the present study is a new flavone glycoside.

EXPERIMENTAL

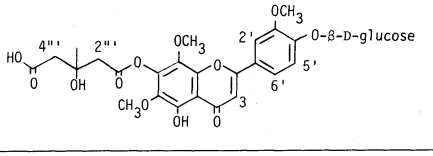
Analytical instruments. Optical rotation was measured with a Japan Spectroscopic DIP-140. FAB-MS spectra were obtained with JEOL JMS-HX 100 and JMA-DA 5000 instruments under xenon bombardment (6.0 keV). UV spectra were taken with a Hitachi 323 visible-ultraviolet autoanalyzer. NMR spectra were measured with a JEOL JNM-FX 200 in DMSO-d_6 with TMS as an internal standard (200 MHz for ^{1}H and 50 MHz for ^{13}C).

Separation and purification. Crude flavonoids were obtained from sudachi peelings by the conventional method.[1] Compound **1** was isolated by repeated chromatography on silica gel, following by gel filtration.

Alkaline hydrolysis of **1**. A mixture of 10 mg of **1** and 10 ml of 0.5 N KOH–EtOH was refluxed for 1 hr, and then neutralized with a 0.5 N HCl aqueous solution. After the mixture had been dried, sudachiin A was isolated by gel filtration.

Acidic hydrolysis of **1**. A mixture of 10 mg of **1** and 2 mg of 0.5N HCL was heated at 90°C for 1 hr, and $CHCl_3$ was then added to the mixture. The $CHCl_3$ layer and aqueous layer were separated with a separating funnel. The $CHCl_3$ solution was rinsed with a little water and then evaporated *in vacuo* to give the aglycone of sudachitin. The aqueous solution was evaporated *in vacuo* to give a glucose.

4′-β-D-glucosylsudachitin 7-O-(3-hydroxy-3-methylglutalate) (**1**). Yellow needles (mp 150～152°C); FAB-MS *m/z* 667 $[M+H]^+$, 689 $[M+Na]^+$; UV λ_{max}^{EtOH} nm: 284, 337; $\lambda_{max}^{AcONa-EtOH.}$ 283, 318, 400 s; $\lambda_{max}^{AlCl_3-EtOH.}$: 295 s, 305, 364 s, 400 s; ^{1}H-NMR $\delta_{ppm}^{DMSO-d_6}$: 1.28 (3H, s, Me), 2.51 (2H, s, $H_{4'''}$), 2.56 (1H, d, $J=14$ Hz, $H_{2'''}$), 2.64 (1H, d, $J=14$ Hz, $H_{2'''}$), 3.78 (3H, s, OMe), 3.88 (6H, s, OMe×2), 5.08 (1H, d, $J=7$ Hz, $H_{1''}$), 6.86 (1H, s, H_3), 7.22 (1H, d, $J=8$ Hz, $H_{5'}$), 7.54 (1H, d, $J=2$ Hz, $H_{2'}$), 7.56 (1H, dd, $J=2,8$ Hz, $H_{6'}$); ^{13}C-NMR $\delta_{ppm}^{DMSO-d_6}$: 27.6 (q, $C_{6'''}$), 45.6, 45.6 (each t, $C_{2'''}$ and/or $C_{4'''}$), 56.1 (q, OMe), 60.3 (q, OMe), 61.3 (q, OMe), 63.5 (t, $C_{6''}$), 70.0 (d, $C_{4''}$), 73.2 (d, $C_{2''}$), 74.1 (d, $C_{3''}$), 76.7 (d, $C_{5''}$), 99.7 (d, $C_{1''}$), 103.2 (s, C_{10}), 103.8 (d, C_3), 110.3 (d, $C_{2'}$), 115.6 (d, C_5), 119.7 (d, $C_{6'}$), 124.6 (s, $C_{1'}$), 128.1 (s, C_8), 131.7 (s, C_6), 145.5 (s, $C_{4'}$), 148.4 (s, $C_{3'}$), 149.4, 149.7



† Studies on Physiologically Active Substances in Citrus Peel. Part VIII.

(each s, C_9 and/or C_7), 151.0 (s, C_5), 163.0 (s, C_2), 170.5, 172.5 (each s, $C_{1'''}$ and/or $C_{5'''}$), 182.4 (s, C_4).

Sudachiin A. Yellow needles (mp 211~213°C; lit.[3] 211~213°C); UV λ_{max}^{EtOH} nm: 283, 335; $\lambda_{max}^{AcONa-EtOH}$: 285, 311 s, 376; $\lambda_{max}^{AlCl_3-EtOH}$: 260, 294, 363; ^{1}H-NMR $\delta_{ppm}^{DMSO\text{-}d_6}$: 3.60 (3H, s, OMe), 3.68 (3H, s, OMe), 3.83 (3H, s, OMe), 4.94 (1H, d, J=7 Hz, $H_{1''}$), 6.37 (1H, s, H_3), 7.14 (1H, d, J=8 Hz, $H_{5'}$), 7.40 (2H, m, $H_{2'}$, $H_{6'}$).

Sudachitin. Yellow needles (mp 239~240°C; lit.[4] 239.5~240.5°C); ^{1}H-NMR $\delta_{ppm}^{DMSO\text{-}d_6}$: 3.76 (3H, s, OMe), 3.86 (6H, s, OME×2), 6.79 (1H, s, H_3), 6.90 (1H, d, J=8 Hz, $H_{5'}$), 7.48 (2H, m, $H_{2'}$, $H_{6'}$).

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