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A NEW FLAVONOL GLUCOSIDE FROM ONION

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Abstract – A new flavonol glucoside was isolated from the outer bulb of onions (*Allium cepa*). The structure was determined by the analysis of spectral data.

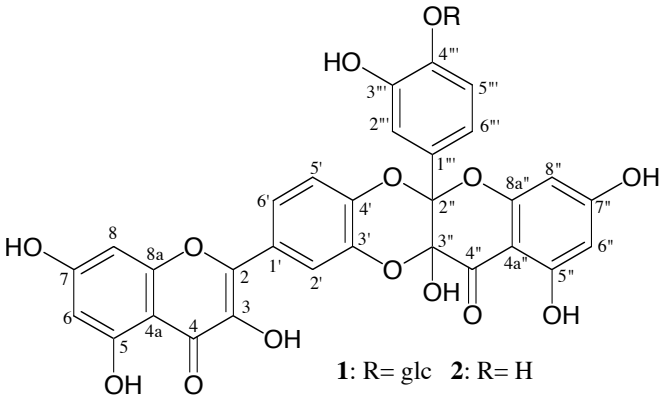

Figure 1

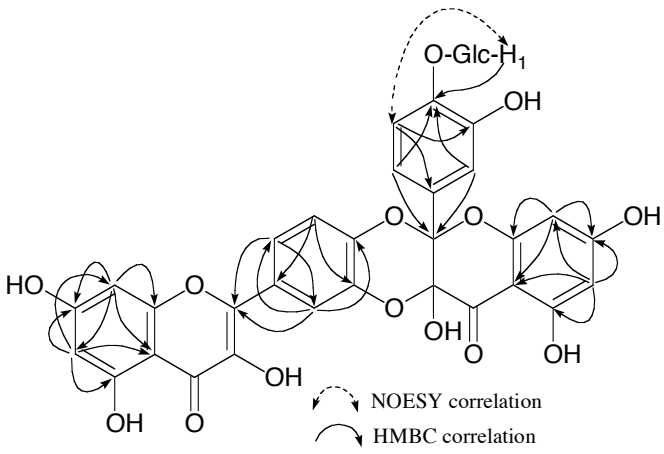

Figure 2

Onion (*Allium cepa*) is well known as a rich source of flavonoid. Hirose *et al*. have been isolated oxidative quercetin derivatives along with quercetin from outer scale of onions.[1] These compounds structurally very interested. For the purpose of utilization of onion and the isolated compounds, we have studied the constituents of *A. cepa*, from which we isolated a flavonol glucoside (**1**) having an oxidative quercetin derivative as aglycone.

The MeOH extract of outer scales of onion was repeatedly purified by Silica gel and LH-20 column chromatography to give a new flavonol glucoside along with three known compounds (**2**, quercetin and quercetin 4'-O-glucoside).

Compound (**1**) was isolated as a yellow amorphous solid, and gave positive reactions to the $FeCl_3$ and Gibbs tests on TLC. In the negative ion FABMS spectrum, the $[M-H]^-$ was observed at *m/z* 763.1143 which correspond to $C_{36}H_{27}O_{19}$. The UV spectrum (250, 270, 304 and 359 nm) showed that **1** had a quercetin-like skeleton. The ^1H and ^{13}C NMR spectra data showed the presence of a β-glucose moiety

and appearance of some splited or broaden signals (shown in EXPERIMENTAL). The split or broaden phenomenon suggested that **1** is a mixture of stereoisomers which gave a single spot on TLC and a single peak on HPLC analysis. The ^{1}H NMR spectrum showed the presence of two sets of *meta* coupled protons (H-6/8 and H-6"/8") and two ABX systems (H-2'/5'/6' and H-2"'/5"'/6"'). The ^{13}C NMR spectra showed the presence of two carbonyl groups (δ 176.8 and 188.6). The ^{1}H and ^{13}C NMR spectra including 2D methods of the aglycone moiety of **1** (Figure 2) was found to be very similar to **2**[1,2] and the enzymatic hydrolysis of **1** gave **2** suggesting **1** to be a glucoside of **2**. The position of the glucose moiety was determined by comparison of the spectral data of the two compounds, the chemical shift of about 0.26 ppm towards low field observed at H-3"' in **1** suggests the glucose moiety is attached at C-4"'. Furthermore, the correlations between H-5"'/glucose H-1(anomeric) in the NOESY spectrum and C-4"'/glucose H-1 in the HMBC spectrum confirmed the position of the glucose molecule at C-4"'.

Hirose *et al*. presumed that oxidative querecetin (**2**) was formed from quercetin by radical scavenging reaction.[1] In a similar presumption **1** might also be produced by same pathway from quercetin and its 4'-O-glucoside.

EXPERIMENTAL

General Method

^{1}H and ^{13}C NMR spectra were measured on AL-300 (JEOL) spectrometer. Chemical shift values were shown as δ values with tetramethylsilane (TMS) as an internal reference. Peak multiplicities are quoted in Hz. Negative FAB-MS spectra were measured on JMS-DX 300 spectrometer equipped with JMA 3500 data analysis system (JEOL). IR spectra was recored on a FT/IR-300 spectrometer(JASCO). UV spectra was recorded on a UV-2200 spectrophotometer (Shimidzu) and optical rotation were measured on P-1020 (JASCO) Polarimeter. HPLC analysis carried out on the Shimadu VP system and Mightysil RP-18 GP (Kanto Chemical Co) as a column. Silica gel 60 (70-230 mesh, Merck), Sephadex LH 20 were used for column chromatography. Kiesel-Gel 60 F_{254} (Merck) was used for analytical TLC. β-Glucosidase for enzymatic hydrolysis was used the products of Oriental Yeast Co Ltd.

Plant Material

Onions were purchased at Gifu City.

Extraction and Isolation

The brownish outer scale of onions (600 g) was air dried, powdered and extracted with MeOH (5 L) at rt for 6 days. The MeOH extract (56 g) was subjected to chromatography on silica gel eluted with $CHCl_3$-MeOH increasing polarity. The $CHCl_3$-MeOH (20:1) fraction was further chromatographed on Sephadex LH 20 eluted with MeOH to yield compound (**2**) (56 mg) and impure quercetin. Finally, quercetin (3.9 g) was purified by the chromatography on Sephadex LH 20 using EtOH-*n*-hexane (9:1) mixture. Fractionation of the $CHCl_3$-MeOH (10:1) fraction by Sephadex LH 20 column eluted with MeOH

yielded a mixture of compound (**1**) and quercetin 4'-O-glucoside. Compound (**1**) (220 mg) and querctin 4'-O-glucoside (2.8 g) were finally obtained by Sephadex LH 20 column chromatography using acetone. Compound (**1**): A yellow amorphous powder; Negative HR-FAB-MS: $[M-H]^-$ *m/z* 763.1143 (Calcd 763.1146 for $C_{36}H_{27}O_{19}$); Negative FAB-MS: *m/z* 763 $[M-H]^-$, 602 $[M-162\ (glc)]^-$; UV λ (nm, MeOH): 250, 270, 304, 359. $[\alpha]_D$ -23° (*c* 0.1, MeOH); IR ν(cm^{-1}, KBr): 3438 (OH), 1646 (carbonyl); ^{1}H NMR (300 MHz, acetone-d_6) δ: 6.04 (br d, *J*= 1.7 Hz, H-6"), 6.11, 6.22 (each br d, *J*= 1.7 Hz, H-8"), 6.27 (d, J= 2.1 Hz, H-6), 6.60 (d, *J*= 2.1 Hz, H-8), 7.17 (d, *J*= 8.8 Hz, H-5"'), 7.20, 7.21 (each d, *J*= 8.8 Hz, H-6"'), 7.29 (d, *J*= 8.8 Hz, H-5'), 7.37, 7.39 (each d, *J*= 1.7 Hz, H-2"'), 7.92 (d, *J*= 2.1 Hz, H-2'), 8.02 (dd, *J*= 8.8, 2.1 Hz, H-6') [aglycone moiety]; δ 4.85, 4.86 (d, *J*= 7.4 Hz, H-1), 3.45-3.52 (m, H-2~6) [glucose moiety]; ^{13}C NMR (75 MHz, acetone-d_6) δ: 145.4 (C-2), 137.6 (C-3), 176.8 (C-4), 104.2 (4a), 162.3 (C-5), 99.3 (C-6), 165.4 (C-7), 94.7 (C-8), 157.9 (C-8a), 127.1 (C-1'), 117.1 (C-2'), 142.9 (C-3'), 141.8 (C-4'), 118.1 (C-5'), 120.6 (C-6'), 101.3 (C-2"), 92.4 (C-3"), 188.6 (C-4"), 100.6 (C-4"a), 165.0 (C-5"), 98.3 (C-6"), 170.2 (C-7"), 97.6 (C-8"), 160.6 (C-8"a), 127.1 (C-1"), 117.2 (C-2"), 148.0 (C-3"'), 147.5 (C-4"'), 117.6 (C-5"'), 120.6 (C-6"') [aglycone moiety]; δ 103.5, 103.4 (C-1), 74.6 (C-2), 77.3 (C-3), 71.0 (C-4), 78.0 (C-5), 62.4 (C-6) [glucose moiety]. All signals were assigned by ^{1}H-^{1}H COSY, HMQC, HMBC spectrum (shown in Figure 2) and comparison with **2**.

Enzymatic hydrolysis of **1**. Compound (**1**) (0.5 mg) and β-glucosidase (16 U) were incubated in a solution (1.0 mL) of citric acid buffer (pH 5.2) at 50° in 3 h. The residue after centrifuge was dissolved in MeOH and the solution was used as analytical sample. HPLC conditions: mobile phase [A: 0.4 % phosphoric acid; B: 0.4 % phosphoric acid-acetinitrile (1 : 1)] 60 min linear gradient (100 % A→100 % B), flow rate: 1.0 mL/min, column oven temp: 40°. *R*t: **1** (40 min), **2** (45 min).

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