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Isolation of a New Quercetin 3-*O*-trisaccharide from the Leaves of *Rubus crataegifolius*

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Abstract – A new quercetin 3-*O*-trisaccharide was isolated from the leaves of *Rubus crataegifolius* (Rosaceae). The structure of this compound was determined as quercetin 3-*O*-α-L-arabinofuranosyl-(1 → 2)-β-D-xylopyranosyl-(1 → 2)-β-D-galactopyranoside on the basis of 2D-NMR spectroscopic data. This flavonol glycoside was isolated for the first time from a natural source.
Keywords – *Rubus crataegifolius*, Rosaceae, flavonol glycoside, quercetin 3-*O*-trisacchride

Introduction

The plant *Rubus crataegifolius* classified into Rosaceae family is widely distributed in mountainous area of Korea (Ko and Jeon, 2003). The fruits are edible and used for treatment of diabetes mellitus, diarrhea, sexual disinclination, amblyopia, and weakness in the folkloric society of Korea (Moon, 1991). The extracts of *R. crataegifolius* roots have been reported to have anti-inflammatory (Cao *et al*., 1996) and apoptosis-inducing activity (Lee *et al*., 2000). In addition, triterpene glycosides have been isolated from *R. crataegifolius* (Jung *et al*., 2001). In the course of our further isolation of constituents from the leaves of *R. crataegifolius*, a new quercetin 3-*O*-trisacchride was isolated and herein reported. Although Gudej (2003) reported the isolation of kaempferol and quercetin glycosides from the leaves of *R. idaeus*; quercetin 3-*O*-β-D-glucoside, quercetin and kaempferol 3-*O*-β-D-galactoside, kaempferol 3-*O*-β-L-arabinopyranoside, kaempferol 3-*O*-β-D-(6"-*E*-*p*-coumaroyl)-glucoside (tiliroside), no flavonoid constituent has been isolated from *R. crataegifolius*.

Experimental

Instrument – Melting point was determined on an Electrothermal 9100 melting point apparatus and was uncorrected. Optical rotation was measured on JASCO DIP-360 digital polarimeter at 25 °C. IR spectrum was measured on a Hitachi 260-01 spectrometer in a KBr disk. FABMS was measured with JEOL JMS DX-300 spectrometer. The ^{1}H-NMR spectrum (δ ppm, *J* in Hz) was recorded in dimethyl sulfoxide-d_6 on a Brucker AM-500 spectrometer (500 MHz), while ^{13}C-NMR spectrum was recorded in the same solvent on a AM-500 instrument at 100 MHz with tetramethylsilane (TMS) as an internal standard.

Plant material – The leaves of *R. crataegifolius* were collected on July in Chiak Mountain, Wonju, Korea and identified by Dr. Sang-Cheol Lim (Prof., Department of Botanical Resources, Sangji University, Korea). The plant collected was dried and crushed for extraction. A voucher specimen (Natchem # 29) was deposited in Laboratory of Natural Product Chemistry, Department of Botanical Resources, Sangji Univeristy, Korea.

Extraction and fractionation – The plant material (2.4 kg) was extracted with each 10 L MeOH under reflux for 5 h three times. The extract was filtered and then concentrated using a rotatory evaporator under reduced pressure to give a viscous mass (156 g) of MeOH extract. This extract was suspended in H_2O, successively fractionated with hexane (0.8 L × 3), $CHCl_3$ (0.8 L × 3), and EtOAc (0.8 L × 3). The residual aqueous layer was then fractionated with 0.8 L BuOH three times and the BuOH-soluble portion was concentrated to give the BuOH fraction (15 g).

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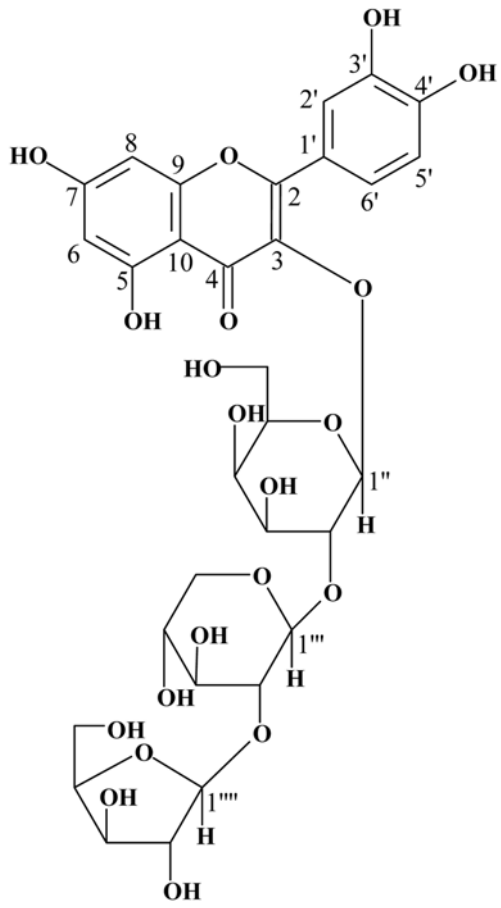

Fig. 1. Structure of compound **1** isolated from *R. crataegifolius*.

Isolation of compound 1 – The BuOH fraction (10 g) was chromatographed on a silica gel column (Art 7734, Merck, Germany, 280 g, 5 × 60 cm) using $CHCl_3$-MeOH (4 : 1) as a mobile phase. The fractions collected by each 80 ml were checked on TLC by 50% sulfuric acid, and then combined into the six fractions (RCB-1 – RCB-6). The fraction RCB-4 (1.2 g) was more purified by Sephadex LH-20 column chromatography and then recrystallized from H_2O to afford compound **1**.

Compound 1 – Yellowish needles, Mg-HCl test: positive, Molisch test: positive; mp 210 - 213 (decomp.), $[\alpha]_D^{20}$ −7.3° (c = 0.96, MeOH); IR ν_{max} (cm^{-1}, KBr): 3,500 - 3,100 (broad, OH), 1,652 (α,β-unsaturated ketone), 1597, 1591, 1483 (aromatic C = C), 1,100 - 1,000 (glycoside); FABMS m/z 751 $[M + Na]^+$; ^{1}H-NMR (DMSO-d_6) δ: Quercetin moiety- 6.20 (1H, br s, H-6), 6.44 (1H, br s, H-8), 6.85 (1H, d, J = 8.0 Hz, H-5'), 7.38 (1H, dd, J = 1.5, 8.0 Hz, H-6'), 8.20 (1H, d, J = 1.5 Hz, H-2'), Sugar moieties- 5.20 (1H, d, J = 7.0 Hz, Gal-1), 3.27 (1H, m, Gal-2), 4.58 (1H, d, J = 6.5 Hz, Xyl-1), 3.23 (1H, m), 5.56 (1H, br s, Ara-1), 4.22 (1H, d, J = 1.5 Hz); ^{13}C-NMR (DMSO-d_6) δ: Table 1.

Table 1. ^{13}C-NMR assignment of compound **1** isolated from *R. crataegifolius*

Carbon	^{13}C	Carbon	^{13}C
Quercetin-1	–	Gal-1"	103.7
2	155.4	2"	77.2
3	134.7	3"	74.8
4	178.1	4"	69.8
5	161.5	5"	74.7
6	99.6	6"	62.0
7	164.1	Xyl-1""	106.0
8	94.3	2""	77.4
9	157.0	3""	71.0
10	104.1	4""	72.3
1'	121.1	5""	66.0
2'	115.7	Ara-1"""	108.4
3'	145.4	2"""	81.6
4'	149.0	3"""	77.3
5'	116.0	4"""	87.0
6'	121.0	5"""	63.3

Acid hydrolysis – Compound **1** (30 mg) dissolved in 50 ml of 10% MeOH was heated under reflux. After cooling, the solution was filtered and then partitioned with ethyl acetate three times. The organic phase was dried *in vacuo* and recrystallized from MeOH to give compound **1a**. Compound **1a** was identified as quercetin by comparison of mixed mp with a standard compound (Sigma, USA). The aqueous layer was neutralized with NH_4OH and then evaporated *in vacuo* to give a residue. The resulting residue was applied to a TLC plate and developed with EtOAc-MeOH-H_2O-AcOH (13 : 6 : 3 : 3). The three R_f values were identical to those of D-galactose, D-xylose and L-arabinose shown by co-TLC.

Results and Discussion

Compound **1** was positive at Mg-HCl test and Molisch test indicating that it belongs to the flavonoid glycoside. IR spectrum of compound **1** showed hydroxyl group of the sugar and phenolic moieties (3,500 - 3,100 cm^{-1}), α,β-unsaturated ketone (1,652 cm^{-1}) and glycoside linkage (1,100 - 1,000 cm^{-1}). These data indicated that compound **1** is a flavonol glycoside. On acid hydrolysis, compound **1** produced quercetin as an aglycone and D-galactose, D-xylose and L-arabinose as sugars. The aglycone was identified by comparison of mixed mp, co-TLC with a standard quercetin (Sigma, USA), whereas the sugars were observed by co-TLC.

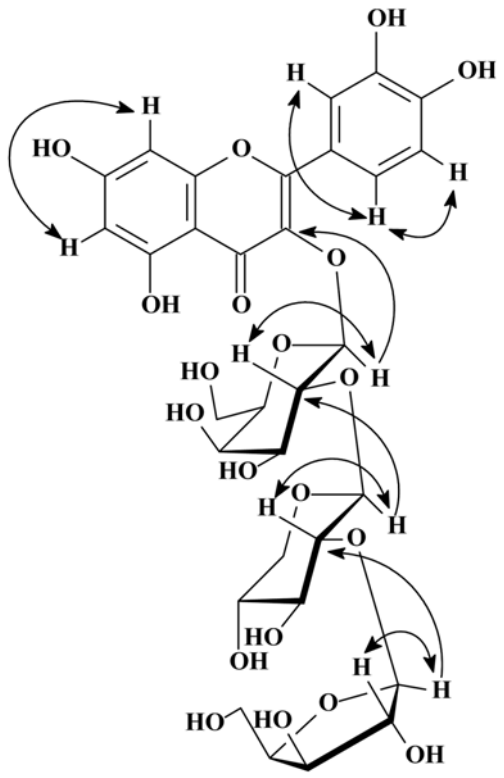

Fig. 2. Selected HMBC correlation in Compound **1**. HMBC (→), COSY(↔).

In the ^{1}H-NMR spectrum of compound **1**, the peaks of H-6 and H-8 of A-ring in quercetin were observed at δ 6.20 and δ 6.44, while the peaks due to H-2', -5', and -6' of those of B-ring were shown at δ 8.20, 6.85 and 7.38. ^{1}H-^{1}H COSY correlations of those peaks, as shown in Fig. 2, evidently represent that the aglycone should be quercetin. Three anomeric protons due to the three sugars were observed at δ 5.20, δ 4.58 and δ 5.56 to which the peaks at δ_C 103.7, 106.0 and 108.4 were connected in the HMQC spectrum, respectively. Thus, compound **1** is quercetin 3-*O*-trisachharide. The terminal sugar was identified as L-arabinofuranose from the five peaks at δ_C 108.4, 81.6, 77.3, 87.0 and 63.3 compared to the literature data (Agrawal, 1989), while D-galactose and D-xylose were not observed as terminal sugars and therefore both should be inner or middle sugars. In the HMBC correlation of compound **1**, δ_H 5.20 due to H-1 of D-galactose was connected to δ_C 134.7 of C-3 in quercetin as shown in Fig. 2, indicating that the inner sugar, D-galactose, was linked to C-3. Therefore, the middle sugar must be D-xylose. In the HMBC correlation, the peak δ_H 5.56 of the anomeric proton of L-arabinofuranose long-range-coupled with δ_C 77.4 of C-2''' of D-xylose, the peak δ_H 4.58 due to H-1 of D-xylose correlated with δ_C 77.2 of C-2" of D-galactose, and δ_H 5.20 of the anomeric proton of D-galactose crossed to the C-3 of the aglycone. In addition, the peak δ_H 3.27 was coupled with δ_H 5.20 in the ^{1}H-^{1}H COSY spectrum and crossed to δ_C 77.2 indicating that the additional sugar was linked to C-2" of the inner sugar D-galactose. Again, the peak δ_H 3.23 (dd-like) was coupled with δ_H 4.58 in the ^{1}H-^{1}H COSY spectrum and further crossed to δ_C 77.4 in the HMQC spectrum, which indicates the terminal sugar D-arabinofuranose was attached at C-2''' of the middle sugar. These correlations were also shown in Fig. 2. Therefore, compound **1** should be quercetin 3-*O*-L-arabinofuranosyl-(1→2)-D-xylopyranosyl-(1→2)-D-galactopyranoside. To find the linkage configuration of the three sugars, coupling constants of the anomeric proton peaks were considered. Since the anomeric proton peak (δ_H 5.56) of L-arabinofuranose was shown to be broad singlet, L-arabinofuranose should pose α-configuration linkage. The coupling constants of anomeric proton peaks of D-xylose and D-galactose were 6.5 Hz and 7.0 Hz indicating that the two sugars have β-configuration linkages. The molecular formula was calculated as $C_{31}H_{36}O_{20}$ from the peak $[M + Na]^+$ (m/z 751) of the FABMS spectrum. The ^{1}H- and ^{13}C-NMR assignment of compound **1** was completed as shown in Table **1**, aided by DEPT, ^{1}H-^{1}H COSY, HMQC, and HMBC spectra. Therefore, the structure of compound **1** was established as quercetin 3-*O*-α-L-arabinofuranosyl-(1→2)-β-D-xylopyranosyl-(1→2)-β-galactopyranoside. Larsen *et al* (1982) have isolated quercetin 3-*O*-β-D-xylopyranosyl (1 → 2)-β-galactopyranoside. However, compound **1** has not been isolated from natural sources.

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