STRUCTURE DETERMINATION OF A VIOLET-BLUE FLOWER FLAVONOID, QUERCETIN 3-GLUCOSYL(1 → 2)GENTIOBIOSIDE FROM PRIMULA POLYANTHA †

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Abstract-----The chemical structure of quercetin 3-glucosyl-(1 → 2)gentiobioside, isolated from the violet blue flowers of Primura polyantha was determined to be quercetin 3-O-(ß-D-glucopyranosyl(1 → 2)-O-ß-D-glucopyranosyl(1 → 6))-ß-D-glucopyranoside unambiguously based on the spectroscopic data.

During the course of our investigation on the blue flower colors due to anthocyanins, we found that the violet-blue pigment of Primura polyantha was mainly produced from hirustidin 3,5-diglucoside and quercetin 3-glucosyl(1 → 2)-gentiobioside (1), where the latter plays an important role in copigmentation with hirustidin 3,5-diglucoside in this plant.[1] Compound (1) was firstly isolated from fresh violet blue flowers of this plant and also from P. sinensis, and its structure was proposed to be quercetin 3-gentiotrioside by Harborne in 1967.[2] Recently, the same quercetin triglucoside was isolated from P. officinalis as a main flavonol glycoside, and its trisaccharide was deduced as one with 1 → 2 and 1 → 6 glycosyl linkages by employing the selective enzymatic hydrolysis of ß-glycosyl bonds by Karl and co-workers in 1981.[3] However, its complete structure including the stereochemistry of the saccharides has not been elucidated yet. We here wish to report the unambiguous structure determination of (1) by modern 2D nmr techniques.[4] Compound (1) consists of one molecule of quercetin and three molecules of glucose, and its molecular weight was confirmed by FABMS exhibiting $M^+ + 1$ ion at m/z 789. Its ^{1}H-nmr spectrum (in DMSO-d_6) showed three characteristic anomeric protons at 5.32, 4.15 and 4.02 ppm, which gave well isolated cross

† Dedicated to the memory of the late Professor Tetsuji Kametani.

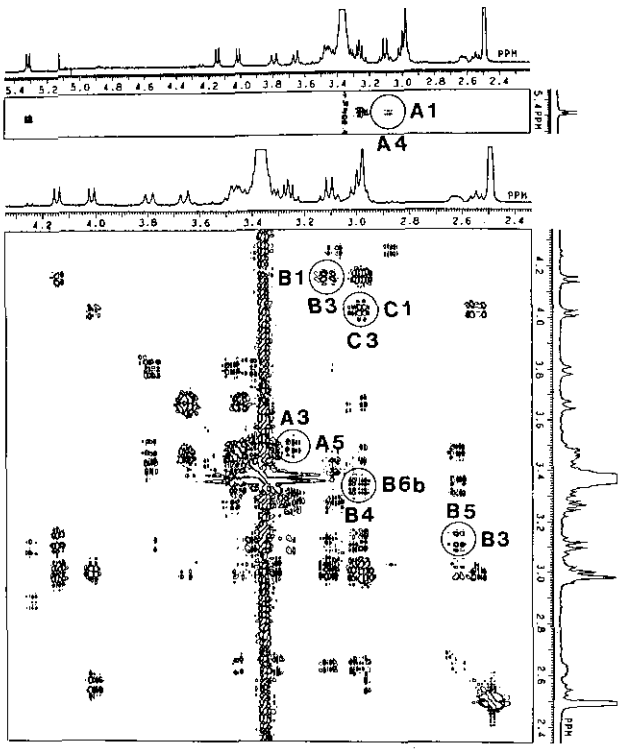

Fig. 1. 500.2 MHz relayed-COSY spectrum of 1 (in DMSO-d_6). The relay cross peaks are labeled using the following notation; the label Xn means the Hn proton of sugar X, e.g., the label B5 means H-5 of sugar B. The label above or below the circle, and the label at the side of the circle indicate the assignments along f_2 (horizontal) and f_1 (vertical) axes, respectively.

peaks at (δ_{H-1}, δ_{H-2}) in 2QF-COSY spectrum. For the clearness of the description, sugar units with cross peaks at (δ_{H-1}, δ_{H-2}) = (5.32, 3.27), (4.15, 2.98) and (4.02, 2.56) are tentatively assigned as sugar A, B and C, respectively. The location of H-3 (3.13 ppm) of sugar B could be identified in Relayed-COSY spectrum (Fig. 1). A spin system attributable to sugar B protons of H-6a (3.46 ppm), H-6b (3.33 ppm), H-5 (2.64 ppm) and H-4 (3.01 ppm) was readily correlated in 2QF-COSY spectrum. The presence of relayed cross peak between H-3 and H-5 completed the assignment of sugar B protons (Fig. 1). The assignments of protons coupled with ^{13}C signals of sugar B were made straightforwardly by ^{13}C - ^{1}H correlation spectrum. The correlation of H-6a (3.80 ppm) with H-6b (3.39 ppm) and H-5 (3.48 ppm) in sugar A could be extended to H-3 (3.26 ppm) through H-4 (3.10 ppm) in 2QF-COSY spectrum. However the higher order coupling hampered the correlation of H-3 of sugar A with H-2 of any sugar unit. The complete assignment of sugar A protons was derived from the observation of ^{13}C - ^{1}H long range correlation by HMBC spectrum,[5] where H-2 (3.27 ppm) and H-3 (3.26ppm) were mutually correlated with C-3 (ca. 76.0 ppm) and C-2 (73.93 ppm), respectively (Fig. 2). After the spectral assignment of sugar A and sugar B, in 2QF-COSY spectrum remained two separate spin systems, one consisting of H-6a (3.67 ppm), H-6b (3.45

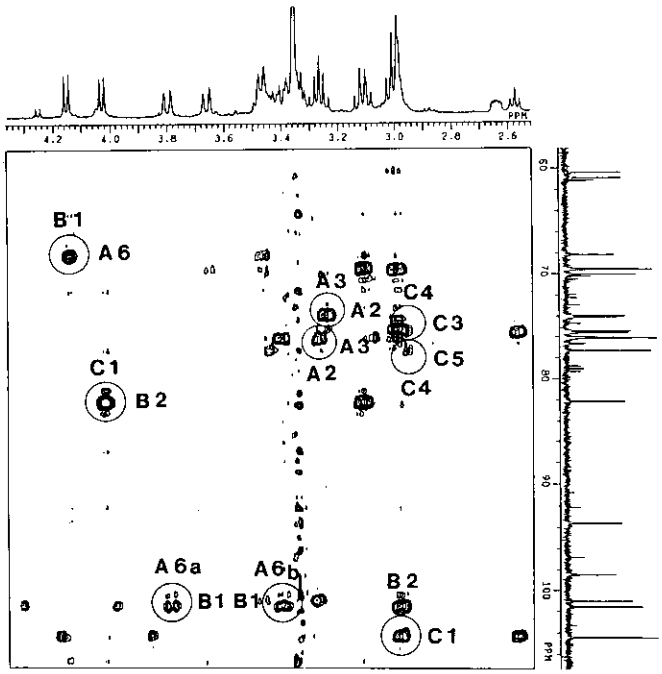

Fig. 2. Trigulcoside region of HMBC spectrum of 1 (in DMSO-d_6). The cross peaks referred in the text are labeled using the same notation as Fig. 1.

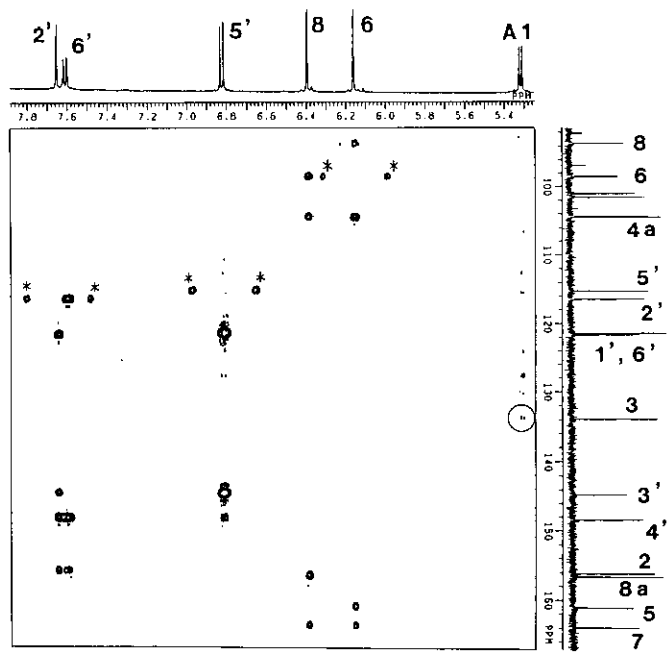

Fig. 3. Quercetin region of HMBC spectrum of 1 (in DMSO-d_6). The direct cross peaks are distinguished by asterisks.

Table 1.
^{1}H-Nmr Spectral Data of
Quercetin 3-glucosyl (1→2) gentiobioside

(500.2 MHz, DMSO-d_6, δ values = ppm)

Aglycone			
H-6	6.16	d	J = 2.1 (Hz)
H-8	6.39	d	J = 2.1
H-2′	7.66	d	J = 2.2
H-5′	6.82	d	J = 8.5
H-6′	7.61	d, d	J = 2.2, 8.5
Glucose-A			
H-1	5.32	d	J = 7.6
H-2*	3.27*	t*	
H-3*	3.26*	t*	
H-4	3.10	t	J = 8.8
H-5	3.48[2*]	m	
H-6a	3.80	d	J = 11.4
H-6b	3.39[3*]	d, d	J = 11.4, 4.4
Glucose-B			
H-1	4.15	d	J = 7.8
H-2	2.98[2*]	m	
H-3	3.13	t	J = 8.6
H-4	3.01	t	J = ∼9
H-5	2.64	d, d, d	J = 9.3, 5.4, 2.2
H-6a	3.46[2*]	m	
H-6b	3.33[3*]	d, d	J = 11.8, 5.4
Glucose-C			
H-1	4.02	d	J = 7.8
H-2	2.56	t	J = 8.2
H-3	2.99[2*]	m	
H-4	2.96[4*]	m	
H-5	2.98[2*]	m	
H-6a	3.67	d	J = 10.7
H-6b	3.45[2*]	m	

*: Values could be exchangeable and assigned by ^{13}C-^{1}H shift correlation.
2*: Assigned by 2QF-COSY.
3*: Assigned by WEFT.
4*: Assigned by HMBC.

Table 2.
^{13}C-Nmr Spectral Data Quercetin
3-glucosyl (1→2) gentiobioside

(125.8 Mz, DMSO-d_6, δ values = ppm)

Aglycone		
C-2	156.00	s
C-3	133.76	s
C-4	177.48	s
C-4a	104.37 or 104.29	s
C-5	160.91	s
C-6	98.42	d
C-7	163.74	s
C-8	93.57	d
C-8a	156.49	s
C-1′	121.51 or 121.29	s
C-2′	116.31	d
C-3′	144.55	s
C-4′	148.21	s
C-5′	115.10	d
C-6′	121.51 or 121.29	d
Glucose-A		
C-1	100.97	d
C-2*	73.92	d
C-3*	76.09 or 76.06	d
C-4	70.01	d
C-5	76.09 or 76.06	d
C-6	68.14	t
Glucose-B		
C-1	101.49	d
C-2	82.12	d
C-3	75.36	d
C-4	69.44	d
C-5	76.00	d
C-6	60.37	t
Glucose-C		
C-1	104.37 or 104.29	d
C-2	74.06	d
C-3	75.54	d
C-4	69.55	d
C-5	77.26	d
C-6	60.89	t

*: Values could be exchangeable.

ppm) and H-5 (2.98 ppm), and the other consisting of H-1 (4.02 ppm), H-2 (2.56 ppm) and H-3 (2.99 ppm). These two spin systems could be combined to complete the assignment of sugar C by the long range correlation of C-3 and C-5 with the same proton resonated at 2.96 ppm, which should be assigned to H-4. The ^{1}H and ^{13}C nmr data are summarized in Tables 1 and 2. The stereochemistry of the sugars was confirmed to be ß-D-glucopyranose from the typical vicinal coupling constants $J_{H-1,\ H-2}$ = ca. 8 Hz and $J_{H-3,\ H-4}$ = ca. 9 Hz (Table 1).

The linkages between sugar and aglycon, and between sugars were determined based on the ^{13}C - ^{1}H long range correlation to avoid the ambiguity inherent in NOE approach.[6] In HMBC spectrum, the anomeric proton of sugar A was long range coupled with C-3 of quercetin, which assignment was derived from ^{13}C-^{1}H long range correlations within the aglycon moiety (Fig. 3), and ^{13}C nmr data for querecetin.[7] The 1 → 6 link between sugar A and B was established based on the long range correlation of H-1 of sugar B with C-6 of sugar A, and the reverse correlations of H-6a and H-6b of sugar A with C-1 of sugar B. The observation of the long range correlation of H-2 of sugar B with C-1 of sugar C and its reverse correlation led to the 1 → 2 link between sugar B and C. The down-field shifts of C-2 of sugar B and C-6 of sugar A are consistent with the established sugar linkages. Finally, the structure of 1 including the stereochemistry of sugars, was established to be 3-O-(ß-D-glucopyranosyl(1 → 2)-O-ß-D-glucopyranosyl(1 → 6)-O-ß-D-glucopyranosyl)-quercetin.

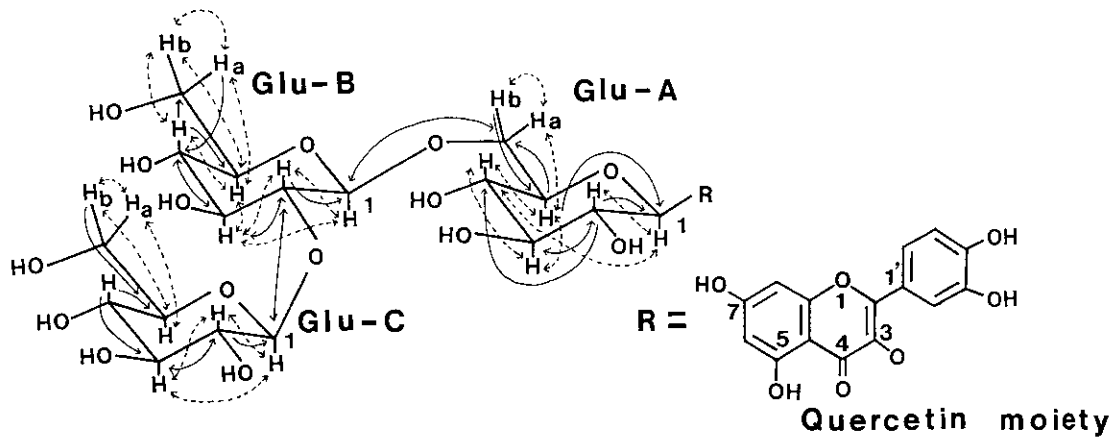

Fig. 4. A summary of ^{1}H - ^{1}H correlations (⟷) by 2QF-COSY and relayed-COSY spectrum, and ^{13}C - ^{1}H long range correlations by HMBC spectrum of 1. The arrow (⟵) points from ^{1}H to ^{13}C. The double-headed arrow (⟷) indicates the position, where the long range correlation was bidirectional.

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