Three New Kaempferol Glycosides from *Cardamine leucantha*

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Three new kaempferol glycosides, kaempferol 3-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-galactopyranosyl-7-*O*-*α*-L-rhamnopyranoside (**1**), kaempferol 3-*O*-*β*-D-galactopyranosyl-7-*O*-*β*-D-glucopyranosyl-(1→3)-*α*-L-rhamnopyranoside (**2**), and kaempferol 3-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-galactopyranosyl-7-*O*-*β*-D-glucopyranosyl-(1→3)-*α*-L-rhamnopyranoside (**3**), were isolated from the whole herbs of *Cardamine leucantha*, along with three known kaempferol glycosides, kaempferol 7-*O*-*α*-L-rhamnopyranoside, kaempferitrin, and kaempferol 3-*O*-*β*-D-galactopyranosyl-7-*O*-*α*-L-rhamnopyranoside. Their structures were elucidated on the basis of spectroscopic methods.

Introduction. – The genus *Cardamine* (Brassicaceae) comprises *ca.* 160 species mainly distributed in the temperate zone, and 39 species and 29 varieties are reported in China [1]. The roots of *Cardamine leucantha* O. E. SCHULZ, locally called '*CaiZiQi*' in Chinese folk medicine, are used for the treatment of respiratory diseases, and the whole plant also can be eaten as wild vegetable or used as a substitute for tea [1][2]. However, very few studies have been published on the chemical constituents or bioactivity of *Cardamine* plants, and the previous studies on this genus mostly focused on glucosinolates [3][4], and there are no previous investigations on the constituents of this species.

Here, we describe the isolation and structure elucidation of three new kaempferol glycosides, including two triosides and one tetraoside, **1**, **2**, and **3** (*Fig. 1*), and the

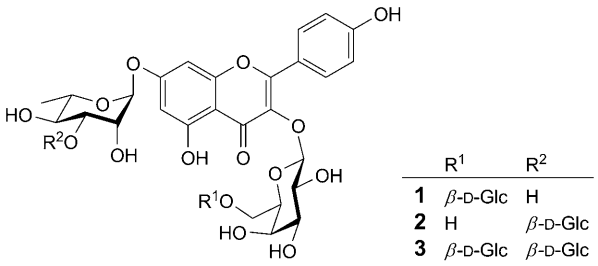

Fig. 1. *The Structures of Compounds* **1**–**3**

isolation of three known kaempferol glycosides from *Cardamine leucantha*, kaempferol 7-*O*-*α*-L-rhamnopyranoside [5], kaempferitrin [5], and kaempferol 3-*O*-*β*-D-galactopyranosyl-7-*O*-*α*-L-rhamnopyranoside [5][6]. All these compounds were reported for the first time from the title plant, and their structures were elucidated based on the combination of 1D- and 2D- (^{1}H- and ^{13}C-NMR, COSY, HSQC, and HMBC) NMR spectroscopic and mass spectrometric (HR-ESI-MS) techniques.

Results and Discussion. – Compound **1** was isolated as a yellow powder. On the basis of the HR-ESI-MS and NMR techniques, a molecular formula of $C_{33}H_{40}O_{20}$ was deduced.

In the aromatic region of the ^{1}H-NMR spectrum (*Table 1*), besides two H-atom signals at δ(H) 6.43 (br. *s*) and 6.80 (br. *s*), an *AA′XX′* spin system was evident as two *doublet*s at δ(H) 8.10 (br. *d*, $J = 8.5$) and 6.85 (br. *d*, $J = 8.5$). The ^{13}C-NMR spectrum (*Table 2*) showed 31 signals for 33 C-atoms (two signals were due to two homotopic C-atoms). The HSQC experiment allowed a complete assignment of all H- and C-atom resonances. The number of the aromatic signals in both the ^{1}H- and ^{13}C-NMR spectra suggested the presence of kaempferol as aglycone [7].

In the upfield region of the spectrum, there were signals due to a saccharidic unit as three anomeric H-atoms (δ(H) 5.54 (br. *s*), 5.37 (*d*, $J = 8.0$), 4.03 (*d*, $J = 7.5$)) and further sixteen saccharidic H-atoms (δ(H) 2.81 to 3.83), suggesting the presence of a trisaccharide unit. Acid hydrolysis afforded glucose, galactose, and rhamnose in agreement with the ^{1}H- and ^{13}C-NMR data. The absolute D-configuration of the glucose and galactose residues, and the L-configuration of the rhamnose unit were based on biogenetic considerations. The *β*-anomeric configurations of the glucose and galactose residues were assigned from the coupling constants of the anomeric H-atoms. The *α*-anomeric configuration of the rhamnose moiety was deduced from the ^{13}C-NMR data. In the ^{13}C-NMR spectrum, the relative upfield shifts of the C(3) and C(7) signals to δ(C) 133.6 and 161.7, respectively, were in agreement with the glycosylation of kaempferol at C(3) and C(7) [7–9]. The sugar sequence was determined on the basis of both 1D- and 2D-NMR, particularly HMBC experiments (*Fig. 2*). The positions of attachment of the glycosyl moieties to the aglycone were deduced by the ^{1}H,^{13}C long-range correlations observed for H–C(1‴) of Rha (δ(H) 5.54)/C(7) of aglycone (δ(C) 161.7), and H–C(1″) of Gal (δ(H) 5.37)/C(3) of aglycone (δ(C) 133.6). The chemical-shift values for the C-atom resonances assigned to the Rha unit were consistent with a terminal rhamnopyranosyl attached directly to the aglycone. On the other hand, the C(6″) (δ(C) 67.2) resonance of the Gal moiety was shifted downfield due to glycosylation and provided the site of attachment of Glc to Gal, which was confirmed by the HMBC correlation from H–C(1‴) of Glc (δ(H) 4.03) to C(6″) of Gal. Thus, the structure of compound **1** was established as kaempferol 3-*O*-*β*-D-glucopyranosyl-(1 → 6)-*β*-D-galactopyranosyl-7-*O*-*α*-L-rhamnopyranoside.

HR-ESI-MS and NMR data indicated that compound **2** had the same molecular formula as **1**. Glucose, galactose, and rhamnose were identified after acid hydrolysis. The differences in the ^{1}H- and ^{13}C-NMR spectra of **1** and **2** suggested that they differ only in the sugar sequence. The ^{1}H- and ^{13}C-NMR chemical shifts, together with COSY, HSQC, and HMBC data, confirmed the identity of the sugars as Glc, Gal, and Rha. The ^{1}H- and ^{13}C-NMR data indicated *β*-anomers for Glc and Gal, and *α*-anomer for Rha.

Table 1. *^{1}H-NMR Data* (500 MHz, DMSO) *of* **1**, **2**, *and* **3**. δ in ppm, J in Hz[a]).

	1	**2**	**3**
Aglycone			
H–C(6)	6.43 (br. *s*)	6.47 (*d*, *J* = 2.0)	6.47 (*d*, *J* = 2.0)
H–C(8)	6.80 (br. *s*)	6.85 (*d*, *J* = 2.5)	6.83 (*d*, *J* = 1.5)
H–C(2′/6′)	8.10 (br. *d*, *J* = 8.5)	8.10 (br. *d*, *J* = 8.5)	8.11 (br. *d*, *J* = 9.0)
H–C(3′/5′)	6.85 (br. *d*, *J* = 8.5)	6.85 (br. *d*, *J* = 9.0)	6.86 (br. *d*, *J* = 9.0)
3-*O*-Gal			
H–C(1″)	5.37 (*d*, *J* = 8.0)	5.42 (*d*, *J* = 7.5)	5.37 (*d*, *J* = 7.5)
H–C(2″)	3.56	3.54	3.56
H–C(3″)	3.39	3.37	3.35
H–C(4″)	3.70	3.65	3.70
H–C(5″)	3.57	3.33	3.57
CH_2(6″)	3.74, 3.41	3.44, 3.29	3.74, 3.42
6″-*O*-Glc			
H–C(1‴)	4.03 (*d*, *J* = 7.5)		4.04 (*d*, *J* = 8.0)
H–C(2‴)	2.82		2.83
H–C(3‴)	2.97		2.97
H–C(4‴)	2.96		2.98
H–C(5‴)	2.89		2.90
CH_2(6‴)	3.55, 3.35		3.55, 3.35
7-*O*-Rha			
H–C(1⁗)	5.54 (br. *s*)	5.58 (br. *s*)	5.57 (br. *s*)
H–C(2⁗)	3.83 (br. *s*)	4.10 (br. *s*)	4.10 (br. *s*)
H–C(3⁗)	3.62	3.76 (*dd*, *J* = 8.5, 2.5)	3.76
H–C(4⁗)	3.30	3.50	3.51
H–C(5⁗)	3.44	3.48	3.48
Me(6⁗)	1.11 (*d*, *J* = 6.5)	1.12 (*d*, *J* = 5.0)	1.13 (*d*, *J* = 5.0)
3⁗-*O*-Glc			
H–C(1⁗′)		4.46 (*d*, *J* = 7.5)	4.47 (*d*, *J* = 7.5)
H–C(2⁗′)		3.08	3.09
H–C(3⁗′)		3.17	3.17
H–C(4⁗′)		3.07	3.08
H–C(5⁗′)		3.16	3.16
CH_2(6⁗′)		3.44, 3.29	3.66, 3.45

[a]) The assignments were based upon COSY, HSQC, and HMBC experiments, with multiplicities and coupling constants in parentheses. Overlapping signals were given without designating multiplicity.

The HMBC experiment allowed us to localize the Gal at C(3), and indicated the presence of a β-D-glucopyranosyl-(1 → 3)-α-L-rhamnopyranoside as disaccharide moiety at C(7). Thus, compound **2** was identified as kaempferol 3-*O*-β-D-galactopyranosyl-7-*O*-β-D-glucopyranosyl-(1 → 3)-α-L-rhamnopyranoside.

Based on NMR and MS results, compound **3** could be assigned to have the molecular formula $C_{39}H_{50}O_{25}$. Besides the H-atoms of a kaempferol moiety, the ^{1}H-NMR spectrum showed four anomeric H-atom signals at δ(H) 5.57 (br. *s*), 5.37 (*d*, *J* = 7.5), 4.47 (*d*, *J* = 7.5), and 4.04 (*d*, *J* = 8.0), as well as signals of 22 saccharidic H-atoms ranging from δ(H) 2.83 to 4.10 ppm, and a *doublet* at δ(H) 1.13 (*d*, *J* = 5.0),

Table 2. *^{13}C-NMR Data* (125 MHz, DMSO) *of* **1**, **2**, *and* **3**. δ in ppm.

	1	**2**	**3**		**1**	**2**	**3**
Aglycone				6″-*O*-Glc			
C(2)	157.0	156.9	157.0	C(1‴)	102.9		102.9
C(3)	133.6	133.5	133.5	C(2‴)	73.2		73.2
C(4)	177.6	177.7	177.6	C(3‴)	76.6		76.5
C(5)	160.9	160.9	160.8	C(4‴)	69.9		69.9
C(6)	99.4	99.5	99.5	C(5‴)	76.5		76.5
C(7)	161.7	161.4	161.4	C(6‴)	60.9		60.9
C(8)	94.6	94.7	94.8	7-*O*-Rha			
C(9)	156.0	155.9	155.9	C(1⁗)	98.5	98.3	98.3
C(10)	105.7	105.7	105.7	C(2⁗)	69.8	69.0	69.0
C(1′)	120.7	120.7	120.7	C(3⁗)	70.3	80.7	80.7
C(2′/6′)	131.1	131.1	131.1	C(4⁗)	71.6	70.5	70.5
C(3′/5′)	115.1	115.1	115.1	C(5⁗)	70.1	69.6	69.6
C(4′)	160.1	160.1	160.1	Me(6⁗)	17.9	17.8	17.8
3-*O*-Gal				3‴″-*O*-Glc			
C(1″)	101.7	101.6	101.6	C(1⁗″)		104.6	104.6
C(2″)	71.1	71.2	71.1	C(2⁗″)		74.0	74.0
C(3″)	72.9	73.1	72.9	C(3⁗″)		76.3	76.2
C(4″)	67.8	67.9	67.8	C(4⁗″)		69.9	69.9
C(5″)	73.9	75.8	73.8	C(5⁗″)		76.7	76.7
C(6″)	67.2	60.2	67.1	C(6⁗″)		61.0	61.0

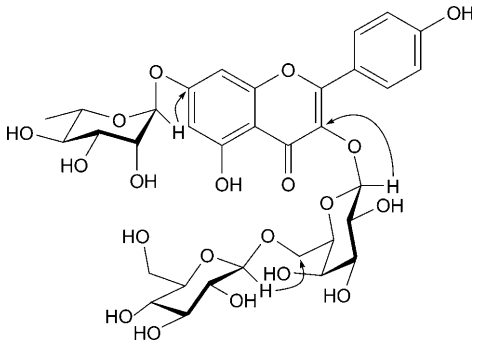

Fig. 2. *Selected HMBCs* (H → C) *of* **1**

which indicated the presence of a tetrasaccharide unit for compound **3**. The ^{1}H- and ^{13}C-NMR spectra of **3** were very similar to those of compound **2**, except for additional signals due to a glucopyranoside moiety. As the CH_2(6″) signals of Gal were deshielded to δ(H) 3.42 and 3.74, C(6″) had to be the attachment position for the second Glc moiety. Accordingly, the C(6″) resonance was shifted downfield to δ(C) 67.1. The site of attachment of the second Glc moiety was also confirmed by a HMBC long-range correlation between the C(1‴) (δ(C) 102.9) of Glc and the CH_2(6″) (δ(H) 3.42 and 3.74) of the Gal unit. The ^{1}H- and ^{13}C-NMR spectra of compound **3** were in full

agreement with the proposed structure. Consequently, the structure of compound **3** was established as kaempferol 3-*O*-*β*-D-glucopyranosyl-(1 → 6)-*β*-D-galactopyranosyl-7-*O*-*β*-D-glucopyranosyl-(1 → 3)-*α*-L-rhamnopyranoside.

The three known compounds, kaempferol 7-*O*-*α*-L-rhamnopyranoside, kaempferol 3-*O*-*β*-D-galactopyranosyl-7-*O*-*α*-L-rhamnopyranoside, and kaempferitrin were identified by comparison of their spectroscopic data with literature values. This is the first report of these compounds from the genus *Cardamine*, except kaempferol 7-*O*-*α*-L-rhamnopyranoside.

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Experimental Part

General. TLC: silica gel GF_{254} plates; spots visualized by UV (254/365 nm) and by spraying with 5% H_2SO_4/EtOH. Column chromatography (CC): silica gel (SiO_2; 100–200 and 200–300 mesh; *Qingdao Marine Chemical Inc.*, Qingdao, P. R. China) and *Sephadex LH-20* (*Pharmacia Biotech AB*, S-Uppsala). M.p.: *XT4A microscope* apparatus; uncorrected. Optical rotations: *Rudolph Research Analytical Autopol II automatic* polarimeter. UV Spectra: *SHIMADZU UV-2450* spectrophotometer. IR Spectra: *Bruker Tensor 27* spectrometer. 1D- and 2D-NMR spectra: *Bruker AV-500* instrument. HR-ESI-MS: *VG ZAB-HS* mass spectrometer.

Plant Material. The whole plant of *Cardamine leucantha* O. E. SCHULZ was collected in September 2006 from Mei County in Shaanxi Province of China, and identified by Prof. *Zhen-Hai Wu*, Northwest A&F University. A voucher specimen (S200608010) was deposited with the School of Pharmaceutical Science and Technology, Tianjin University, P. R. China.

Extraction and Isolation. The air-dried plant material of *Cardamine leucantha* O. E. SCHULZ (14 kg) was extracted with 91% EtOH (50 l, 14 d) at r.t., and then refluxed once with 90% EtOH (2 h) and twice with 60% EtOH (2 h). The solvent was evaporated under reduced pressure to give a dark residue (1600 g), which was suspended in H_2O (6 l) and then sequentially extracted with petroleum ether (PE; 12 l), $CHCl_3$ (12 l), AcOEt (18 l), and BuOH (35 l). The AcOEt extract (11.8 g) was subjected to CC (SiO_2; gradient CH_2Cl_2/MeOH 93 : 7, 8 : 2) to give 68 fractions. *Frs. 24–31* (10.2 g) were combined and further submitted to CC (SiO_2; AcOEt/MeOH/H_2O 98 : 2 : 1) to give kaempferol 7-*O*-*α*-L-rhamnopyranoside (52.5 mg).

The BuOH extract (237 g) was subjected to *D101* macroporous-resin CC and eluted with H_2O, followed by increasing concentrations of EtOH in H_2O (30% EtOH, 50% EtOH, 70% EtOH, 90% EtOH) as eluent to yield five fractions. The fraction eluted with 30% EtOH was then applied to repeated CC (SiO_2; gradient AcOEt/MeOH/H_2O 93 : 7 : 2, 9 : 1 : 0.75, 88 : 12 : 7.5) to give 33 fractions. *Frs. 11–14* were combined and further purified by repeated recrystallization (MeOH) to yield kaempferol 3-*O*-*β*-D-galactopyranosyl-7-*O*-*α*-L-rhamnopyranoside (200 mg). *Frs. 21–25* (8.5 g) were combined and further purified by CC (SiO_2; AcOEt/MeOH/H_2O 88 : 12 : 5) and *Sephadex LH-20* (MeOH) to give compounds **1** (100 mg) and **2** (60 mg). *Frs. 26–28* (7.6 g) were combined and further purified by CC (SiO_2; AcOEt/MeOH/H_2O 86 : 14 : 5) and *Sephadex LH-20* (MeOH) to give compound **3** (50 mg). The 50% EtOH fraction was subjected to CC (SiO_2; gradient AcOEt/MeOH/H_2O 97 : 3 : 2, 9 : 1 : 0.5, 85 : 15 : 5) to afford 38 fractions. *Fr. 6* was loaded on a SiO_2 column and eluted with AcOEt/MeOH/H_2O 9 : 1 : 1 to give kaempferitrin (20 mg).

Acid Hydrolysis. Compounds **1** (3.0 mg) and **2** (20.1 mg) were dissolved in 1 ml and 2 ml of 1M HCl (H_2O/dioxane 1 : 1) separately and heated in a H_2O bath at 80° for 2 h, then the dioxane was evaporated, and the aglycone was removed by extracting with AcOEt (5 × 2 ml). The aq. layer was neutralized with Ag_2CO_3, centrifuged, and evaporated to dryness. The monosaccharides were identified as Glc, Rha, and Gal by SiO_2 TLC (AcOEt/MeOH/H_2O/AcOH 13 : 2 : 1 : 3) comparison with authentic sugars.

*Kaempferol 3-*O*-β-*D*-Glucopyranosyl-(1 → 6)-β-*D*-galactopyranosyl-7-*O*-α-*L*-rhamnopyranoside* (= *7-[(6-Deoxy-α-L-mannopyranosyl)oxy]-5-hydroxy-2-(4-hydroxyphenyl)-4-oxo-4*H*-chromen-3-yl 6-*

O-*β*-D-*Glucopyranosyl-β*-D-*galactopyranoside*; **1**). Pale yellow powder. M.p. 204–206°. $[\alpha]_D^{25} = -156.5$ ($c = 0.621$, MeOH). UV (MeOH): 268 (4.39), 346 (4.30). IR (KBr): 3385, 2934, 2108, 1658, 1597, 1545, 1509, 1489, 1461, 1413, 1350, 1286, 1209, 1180, 1075, 1008, 959, 806. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*, resp. HR-ESI-MS: 779.2001 ($[M + Na]^+$, $C_{33}H_{40}NaO_{20}^+$; calc. 779.2005).

Kaempferol 3-O-*β*-D-*Galactopyranosyl-7*-O-*β*-D-*glucopyranosyl-(1 → 3)-α*-L-*rhamnopyranoside* (= *7-[[6-Deoxy-3*-O-*(β*-D-*glucopyranosyl)-α*-L-*mannopyranosyl]oxy}-5-hydroxy-2-(4-hydroxyphenyl)-4-oxo-4*H-*chromen-3-yl β*-D-*Galactopyranoside*; **2**). Pale yellow powder. M.p. 200–202°. $[\alpha]_D^{27} = -121.1$ ($c = 1.024$, MeOH). UV (MeOH): 267 (4.45), 348 (4.32). IR (KBr): 3420, 2937, 2830, 1654, 1598, 1490, 1456, 1353, 1339, 1286, 1276, 1207, 1178, 1068, 1012, 961, 807. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*, resp. HR-ESI-MS: 779.1997 ($[M + Na]^+$, $C_{33}H_{40}NaO_{20}^+$; calc. 779.2005)

Kaempferol 3-O-*β*-D-*Glucopyranosyl-(1 → 6)-β*-D-*galactopyranosyl-7*-O-*β*-D-*glucopyranosyl-(1 → 3)-α*-L-*rhamnopyranoside* (= *7-[[6-Deoxy-3*-O-*(β*-D-*glucopyranosyl)-α*-L-*mannopyranosyl]oxy}-5-hydroxy-2-(4-hydroxyphenyl)-4-oxo-4*H-*chromen-3-yl 6*-O-*β*-D-*Glucopyranosyl-β*-D-*galactopyranoside*; **3**). Pale yellow powder. M.p. 207–209°. $[\alpha]_D^{27} = -57.3$ ($c = 1.012$, H_2O). UV (MeOH): 268 (4.31), 347 (4.21). IR (KBr): 3383, 2888, 1657, 1599, 1492, 1453, 1352, 1284, 1209, 1177, 1074, 1015. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*, resp. HR-ESI-MS: 941.2898 ($[M + Na]^+$, $C_{39}H_{50}NaO_{25}^+$; calc. 941.2897).

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