Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research

J. Chem. Pharm. Res., 2011, 3(6):799-804

ISSN No: 0975-7384
CODEN(USA): JCPRC5

Kaempferol glycosides from *Siraitia grosvenorii*

Venkata Sai Prakash Chaturvedula* and Indra Prakash

Organic Chemistry Department, The Coca-Cola Company, Global Research and Development, One Coca-Cola Plaza, Atlanta, GA 30313, USA

ABSTRACT

Two kaempferol glycosides were isolated from the purification of the commercial extract of Luo Han Go (Siraitia grosvenorii) on a C-18 column using a Biotage Flash Chromatography system. The structures of the isolated compounds were characterized as kaempferol-3-O-α-L-rhamnoside and kaempferol-3,7-O-α-L-dirhamnoside on the basis of extensive spectral and chemical studies. The complete ^{1}H and ^{13}C NMR spectral assignments of the two isolated compounds are reported herewith on the basis of 1D (^{1}H and ^{13}C) and 2D (COSY, HSQC, HMBC) NMR and high resolution mass (HRMS) spectroscopic data. The aglycone and sugar residues were identified on the basis of hydrolysis studies.

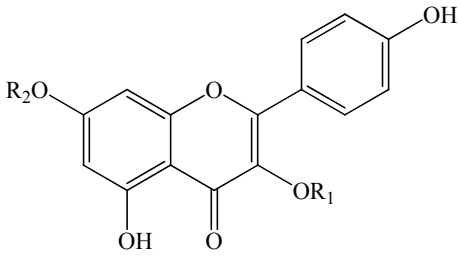
Keywords: *Siraitia grosvenorii*, Curcubitaceae, Luo Han Go, Kaempferol glycosides, NMR, MS, Chemical studies.

INTRODUCTION

The fruit of *Siraitia grosvenorii* (Swingle) Lu & Zhang (*Momordica grosvenorii*; Cucurbitaceae) also known as Luo Han Guo grows in Guangxi, People's Republic of China, and is used as an expectorant as well as a natural sweetener in that country [1-3]. The fruit has been used for centuries in traditional Chinese medicine for the treatment of pulmonary demulcent and emollient for the treatment of dry cough, sore throat, and constipation [4]. Luo Han Guo is well known now throughout the world due to its intense sweet taste and has been used as a non-caloric natural sweetener in some countries. Previous chemical investigation of this species resulted in the isolation of several triterpenoid glycosides also known as mogrosides [1-4]. Some of the compounds among them are sweet such as mogroside V and mogroside IVa and their relative sweetness was about 250-300 times to that of sucrose.

In continuation of our study on the isolation of natural sweeteners from the commercial extracts of *S. rebaudiana* [5-9], we have recently reported the isolation and characterization of six

cucurbitane glycosides namely mogroside IVa, mogrosides V & VI, isomogroside V, 11-oxomogroside V and siamenoside I from the aqueous alcoholic extract of Luo Han Go extract based on the extensive NMR and Mass spectroscopic studies [10]. In this paper we are describing the isolation and purification of two flavonoid glycosides namely kaempferol-3-*O*-α-L-rhamnoside (**1**) and kaempferol-3,7-*O*-α-L-dirhamnoside (**2**) that were characterized on the basis of COSY, HSQC, HMBC and NOESY spectral data.



1; R_1 = α-L-rhamnopyranosyl; R_2 = H

2; $R_1 = R_2$ = α-L-rhamnopyranosyl

3; $R_1 = R_2$ = H

Figure 1: Structures of **1**-**2** and kaempferol (**3**)

EXPERIMENTAL SECTION

General Methods

NMR spectra were acquired on a Varian Unity Plus 600 MHz instrument using standard pulse sequences at ambient temperature. Chemical shifts are given in δ (ppm), and coupling constants are reported in Hz. IR spectral data was acquired using a Perkin Elmer 400 Fourier Transform Infrared (FT-IR) Spectrometer with Universal attenuated total reflectance (UATR) polarization accessory. HRMS data was generated with a Thermo LTQ Orbitrap Discovery mass spectrometer in the positive ion mode electrospray. Instrument was mass calibrated with a mixture of Ultramark 1621, MRFA [a peptide], and caffeine immediately prior to accurate mass measurements of the samples. Samples were diluted with water:acetonitrile:methanol (1:2:2) and prepared a stock solution of 50 ul concentration for each sample. Each sample (25 ul) was introduced via infusion using the on-board syringe pump at a flow injection rate of 120 ul/min. Low pressure chromatography was performed on a Biotage Flash system using a C-18 cartridge (40+ M, 35-70 μm). TLC was performed on Baker Si-C_{18}F plates with mobile phase H_2O-MeOH (35:65). Identification of the spots on the TLC plate was carried out by spraying 10% H_2SO_4 in EtOH and heating the plate at about 80^o C. Analytical HPLC for sugar analysis was carried out with a Waters 600E multisolvent delivery system using a Phenomenex Luna C_{18} non-chiral (150 x 4.6 mm, 5 μm) column.

Materials

The Luo Han Guo commercial extract was purchased from Chengdu Biopurify Phytochemicals, China. A voucher specimen is deposited at The Coca Cola Company, No. VSPC-3166-99.

Isolation and Purification

The Luo Han Guo extract (50 g) was purified on a C-18 column using a Biotage Flash chromatography system (Solvent system: gradient from 20-80% MeOH-water, 60 mL/min. Detection at UV 210 nm). Fractions 21-38 furnished the earlier describe compounds namely mogroside IVa, mogrosides V & VI, isomogroside V, 11-oxomogroside V and siamenoside I [10]. Fractions 39-47 (2.4 g) were combined and further subjected to repeated flash chromatography purification with gradient (Solvent system: 50% MeOH-water to 75% MeOH-water, 20 mL/min, Detection: UV 210 nm) yielded two glycosides Kaempferol-3-O-α-L-rhamnoside (**1**. 12.4 mg) and kaempferol-3,7-O-α-L-dirhamnoside (**2**, 8.6 g).

*Kaempferol-3-O-α-L-rhamnoside (**1**):* Pale yellow powder, IR ν_{max}: 3365, 2935, 1655, 1610, 845 cm^{-1}; ^1H NMR (600 MHz, C_5D_5N, δ ppm) and ^{13}C NMR (150 MHz, C_5D_5N, δ ppm) spectroscopic data see Table 3; HRMS *m/z* $[M+Na^+]$ calcd for $C_{21}H_{20}O_{10}Na$: *m/z* 455.0954; found 455.0962.

*Kaempferol-3,7-O-α-L-dirhamnoside (**2**):* Light yellow crystals, IR ν_{max}: 3357, 2932, 1658, 1615, 855 cm^{-1}; ^1H NMR (600 MHz, C_5D_5N, δ ppm) and ^{13}C NMR (150 MHz, C_5D_5N, δ ppm) spectroscopic data see Table 1; HRMS *m/z* $[M+Na^+]$ calcd for $C_{27}H_{30}O_{14}Na$: *m/z* 601.1533; found 601.1542.

*Identification of aglycone moiety in **1-2**:*

A solution of each compound **1** and **2** (2 mg) dissolved in MeOH was heated to reflux with 2 ml of sulphuric acid under stirring. Reflux continued until the absence of starting material (about 6 h). The reaction mixture was concentrated under vacuum in a rotavaporator and suspended in 20 ml water. Extracted three times with ethyl acetate (3 x 20 ml) and the combined organic layer was washed with water and dried over anhydrous $MgSO_4$. The ethyl acetate layer was filtered over celite and concentrated under vacuum to yield a compound which was identified as kaempferol (**3**) by comparison of its co-TLC with standard compound and NMR spectral data [11-13]. Same aglycone was obtained for both compounds **1** and **2**.

*Determination of sugar configuration in **1-2**:*

Each compound **1** and **2** (1 mg) was hydrolysed with 2 M HCl (2 mL) for 18 h. After cooling, the mixture was diluted with 5 ml water, passed through an Amberlite IRA400 column, lyophilized and the residues obtained were converted to the corresponding thiocarbamoyl-thiazolidine carboxylate derivative with L-cysteine methyl ester and *O*-tolyl isothiocyanate as described above. The sugars were identified as L-rhamnose (*t*R, 21.32 min) from the hydrolysis experiments with **1** and **2** [authentic samples: D- rhamnose (*t*R, 11.73 min) and L- rhamnose (*t*R, 21.64 min)] [12].

RESULTS AND DISCUSSION

Compound **1** was obtained as an amorphous powder and its molecular formula was established as $C_{21}H_{20}O_{10}$ from its HRMS spectral data that showed $[M+Na]^+$ ion at *m/z* 455.0962; this was supported by the ^{13}C NMR spectral data. The UV spectrum of **1** showed λ max at 263, 317, and 345 nm suggested a flavonoid structure [11-13]. The ^1H NMR spectrum of **1** showed the presence of two meta-coupled aromatic protons at δ 6.28 and 6.74 corresponds to H-6 and H-8 protons appeared separately as doublets having coupling constants 1.8 and 2.1 Hz respectively. In addition, the ^1H NMR spectrum of **1** also showed the presence of two doublet of doublets at δ 7.02 and 7.83 with coupling constants 9.0/1.8 Hz and 8.9/1.8 Hz respectively corresponds to the 4 aromatic protons of ring B; characteristic for the 3,5,7,4'-tetrasubstituted flavone. The ^1H NMR

of **1** also showed the presence of an anomeric proton as a doublet at δ 5.40 suggesting a sugar residue in its structure which was identified as L-rhamnosyl moiety on the basis of acid hydrolysis and by preparing the corresponding thiocarbamoyl-thiazolidine carboxylate derivative with L-cysteine methyl ester and *O*-tolyl isothiocyanate, and in comparison of its retention time with the standard sugars as described in the literature [14]. Sulphuric acid hydrolysis of **1** yielded a compound which was identified as kaempferol (**3**) by comparison of its co-TLC with standard compound and NMR spectral data [11-13]. The presence of L-rhamnosyl moiety was further supported by the presence of a secondary methyl group as a doublet at δ 0.94 (*J*=5.7 Hz) as well as the absence of oxymethylene group at C-5 position of the sugar unit. The anomeric proton had a coupling constant of 1.8 Hz, similar to rebaudioside C isolated from *S.rebaudiana* [15] confirming the α-orientation of L-rhamnosyl moiety. The ^{1}H and ^{13}C NMR values for all the carbons were assigned on the basis of HSQC and HMBC correlations (Table 1).

Table 1. ^{1}H and ^{13}C NMR chemical shift values for compounds 1 and 2 in C_5D_5N [a-c]

Position	1		2	
	δ_H	δ_C	δ_H	δ_C
2		158.2		157.8
3		136.1		136.0
4		179.0		179.2
5		162.3		162.6
6	6.27 d, 1H, 2.1	99.7	6.28 d, 1H, 1.8	99.9
7		162.8		165.6
8	6.72 d, 1H, 2.1	94.7	6.74 d, 1H, 2.1	94.5
9		157.2		157.6
10		107.1		105.5
1'		121.4		121.7
2'	7.81 dd, 1H, 9.1, 1.8	131.3	7.83 dd, 1H, 8.9, 1.8	131.5
3'	6.98 dd, 1H, 8.9, 2.1	116.6	7.02 dd, 1H, 9.0, 1.8	116.4
4'		161.6		161.8
5'	6.98 dd, 1H, 8.9, 2.1	116.6	7.02 dd, 1H, 9.0, 1.8	116.4
6'	7.81 d, 1H, 9.1, 1.8	131.5	7.83 dd, 1H, 8.9, 1.8	131.6
Sugar I				
1"	5.42 d, 1H, 1.8	104.0	5.40 d, 1H, 1.8	103.8
2"	4.22 m, 1H	72.2	4.24 m, 1H	71.6
3"	3.73 m, 1H	72.5	3.75 m, 1H	72.4
4"	3.36 m, 1H	73.7	3.35 m, 1H	73.4
5"	3.38 m, 1H	71.4	3.38 m, 1H	71.4
6"	0.93 d, 3H, 5.7	18.4	0.94 d, 3H, 5.7	18.6
Sugar II				
1"'	5.57 d, 1H, 1.8	100.5		
2"'	4.13 m, 1H	71.7		
3"'	3.78 m, 1H	72.3		
4"'	3.46 m, 1H	73.2		
5"'	3.54 m, 1H	71.3		
6"'	1.21 d, 3H, 5.7	18.7		

[a] assignments made on the basis of COSY, HSQC and HMBC correlations; [b] Chemical shift values are in δ (ppm); [c] Coupling constants are in Hz.

Based on the above spectral and chemical studies it was suggested that compound **1** is having flavonoid skeleton [16] of kaempferol as an aglycone moiety and α-L-rhamnoside as a sugar unit. The placement of the L-rhamnosyl moiety was identified at C-3 position on the basis of key COSY and HMBC correlations as shown in Figure 2. Thus, based on the above spectral data, structure of **1** was assigned as kaempferol-3-*O*-α-L-rhamnoside consistent to the reported literature values [12].

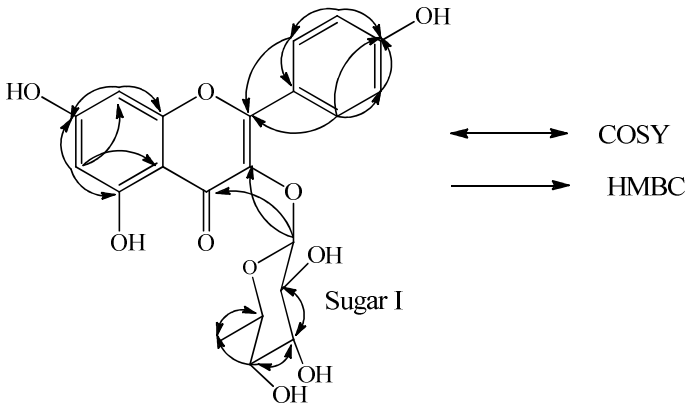


Figure 2: Key COSY and HMBC correlations of **1**

Compound **2** was also obtained as an amorphous powder and its molecular formula was established as $C_{27}H_{30}O_{14}$ from its HRMS spectral data which showed $[M+Na]^+$ ion at *m/z* 601.1542. The UV spectrum of **2** also showed λ max at 265, 318, and 343 nm suggested a flavonoid structure similar to **1** and as reported in the literature [17-18]. The ^{1}H NMR spectrum of **2** showed the presence of two meta coupled aromatic protons at δ 6.27 and 6.72 corresponds to H-6 and H-8 protons, two doublet of doublets at δ 6.98 and 7.81 for H-3'/H-5' and H-2'/H-6' protons of ring B; characteristic for the 3,5,7,4'-tetrasubstituted flavone as in **1**. Sulphuric acid hydrolysis of **2** yielded kaempferol (**3**) suggesting similar aglycone moieties in **1** and **2**. The ^{1}H NMR spectrum of **2** showed the presence of two anomeric protons at δ 5.42 and 5.57 as doublets suggesting the presence of two sugar units in its structure which were identified as L-rhamnosyl moieties on the basis of acid hydrolysis and by preparing the corresponding thiocarbamoyl-thiazolidine carboxylate derivative with L-cysteine methyl ester and *O*-tolyl isothiocyanate as in **1**. The anomeric protons had a coupling constants of 1.8 Hz, confirming their α-orientation of L-rhamnosyl moieties. A close comparison of the ^{1}H and ^{13}C NMR data of **1** and **2** suggested the placement of one L-rhamnosyl moiety at C-3 position of the basic flavone moiety [19], leaving the assignment of the additional L-rhamnosyl unit. The placement of the second L-rhamnosyl unit was established at C-7 position on the basis of key HMBC correlations: H-6/C-5, C-7, C-8; H-8/C-6, C-7, C-9, C-10 and H-1'''/C-7, C-2''', C-3'''. The ^{1}H and ^{13}C NMR values for all the carbons were assigned on the basis of HSQC and HMBC correlations and are given in Table 1. The structure was further supported by the COSY and HMBC correlations as shown in Figure 3.

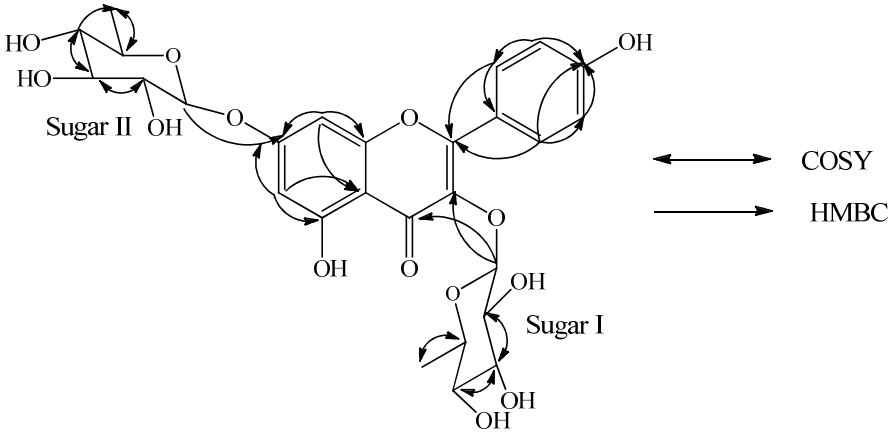


Figure 3: Key COSY and HMBC correlations of **2**

From the above spectral and chemical studies, structure of **2** was unambiguously assigned as kaempferol-3,7-O-α-L-dirhamnoside [11, 13, 20].

CONCLUSION

Two known kaempferol glycosides were isolated from the commercial extract o of *Siraitia grosvenorii* obtained from Chengdu Biopurify Phytochemicals Limited, China. The structures of the isolated compounds were identified as kaempferol-3-*O*-α-L-rhamnoside (**1**) and kaempferol-3,7-*O*-α-L-dirhamnoside (**2**) on the basis of spectroscopic and chemical studies as well as by comparing their physical and spectral properties reported in the literature. The complete ^{1}H and ^{13}C NMR spectral assignments of the two isolated compounds are reported herewith in CD_5N_5 based on 1D and 2D NMR, and HRMS data as well as chemical studies.

REFERENCES

[1] T Takemoto; S Arihara; T Nakajima; M Okuhira, *Yakugaku Zasshi*, **1983**, 103, 1151-1154.
[2] T Takemoto; S Arihara; T Nakajima; M Okuhira, *Yakugaku Zasshi*, **1983**, 103, 1155-1166.
[3] T Takemoto; S Arihara; T Nakajima; M Okuhira, *Yakugaku Zasshi*, **1983**, 103, 1167-1173.
[4] AS Yasushi; M Yuji; I Hiroshi; S Masaki; N Yoshihisa, *J. Agric. Food Chem*., **2005**, 53, 2941-2946.
[5] VSP Chaturvedula; U Mani; I Prakash, *Molecules*, **2011**, 16, 3552-3562.
[6] VSP Chaturvedula; I Prakash, *Molecules*, **2011**, 16, 2937-2943.
[7] VSP Chaturvedula; I Prakash, *Carbohydr. Res*., **2011**, 346, 1057-1060.
[8] VSP Chaturvedula; J Rhea; D Milanowski; U Mocek; I Prakash, *Nat. Prod. Commun*., **2011**, 6, 175-178.
[9] VSP Chaturvedula; I Prakash, *Nat. Prod. Commun*., **2011**, 6, 1059-1062.
[10] VSP Chaturvedula; I Prakash, *J. Carb. Chem*., **2011**, 30, 16-26.
[11] K Nakano; M Takatani; T Tomimatsu; T Nohara, *Phytochemistry*, **1983**, 22, 2831-2833.
[12] G Toker; M Memisoglu; E Yesilada; M Aslan, *Turk. J. Chem*., **2004**, 28, 745-749.
[13] PE Granja-Perez; MM Gamboa-Angulo; F Escalante-Erosa; LM Pena-Rodriguez, *J. Mex. Chem. Soc*., **1999**, 43, 188-191.
[14] T Tanaka; T Nakashima; T Ueda; K Tomii; I Kouno, *Chem. Pharm. Bull*., **2007**, 55, 899-901.
[15] I Sakamoto; K Yamasaki; O Tanaka, *Chem. Pharm. Bull*.. **1977**, 25, 844-846.
[16] N Savitramma; ML Rao; G Bhumi, *J. Chem. Pharm. Res*., **2011**, 3, 28-34.
[17] S Arora; S Vijay; D Kumar, *J. Chem. Pharm. Res*., **2011**, 3, 145-150.
[18] RK Parabathina; E Muralinath; PL Swamy; VVSN Harikrishna; KS Sree, *J. Chem. Pharm. Res*., **2011**, 3, 816-834.
[19] DE Okwu; FU Nnamdi, *J. Chem. Pharm. Res*., **2011**, 3, 1-10.
[20] VH Barve; SN Hiremath; SR Pattan; SC Pal, *J. Chem. Pharm. Res*., **2011**, 2, 200-209.