An Additional Sweet Dihydroflavonol Glycoside from Leaves of *Engelhardtia chrysolepis*, a Chinese Folk Medicine, Huang-qi

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From leaves of *Engelhardtia crysolepis* HANCE (Juglandaceae), which have been used as a sweet tea in China, we have isolated a sweet dihydroflavonol glycoside, and the structure was elucidated on the basis of chemical and spectral evidence as 3-*O*-β-D-glucopyranosyl(1→3)-α-L-rhamnopyranosyl-(2*R*,3*R*)-taxifolin.

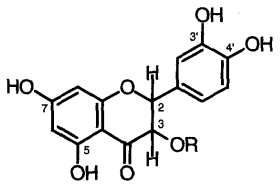
Keywords *Engelhardtia crysolepis;* Juglandaceae; Chinese folk medicine; dihydroflavonol glycoside; sweet glycoside; taxifolin glycoside; huangqioside E; neohuangqioside E; huang-qi

The leaves of *Engelhardtia crysolepis* HANCE (Juglandaceae) have been used as a sweet tea called huang-qi in China. In a preceding paper,[1] we reported on the isolation of neoastilbin (3-*O*-α-L-rhamnosyl-(2*S*,3*S*)-taxifolin, **1**) as a sweet principle of the leaves together with three non-sweet isomers of **1**, astilbin (**2**), neoisoastilbin (**3**) and isoastilbin (**4**). In further investigation of the sweet principle of the leaves, we isolated an additional new sweet dihydroflavonol glycoside (**5**) named huangqioside E. This paper deals with the structural elucidation and isomerization of **5**.

A methanolic extract of the leaves was defatted with ether and then repeatedly chromatographed to give a sweet compound (**5**) as colorless needls in a yield of 0.036%, $[\alpha]_D^{25}$ −20° (c=0.2, EtOH).

The ^{1}H- and ^{13}C-nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectra revealed that **5** was a taxifolin glycoside having two monosaccharide units. Compound **5** afforded D-glucose and L-rhamnose after acid hydrolysis.[2] The circular dichroism (CD) spectrum of **5** showed almost the same curve to that of **2**, demonstrating that the aglycone of **5** is (2*R*,3*R*)-taxifolin (**6**). In a comparison of the ^{13}C-NMR spectrum of **5** with that of **6**, a glycosylation shift was observed at C-2, -3, -4 of the aglycone moiety of **5**,[3] indicating that **5** was a 3-*O*-glycosylated compound of **6**. The structure of the sugar moiety was determined as follows. The assignment of the proton signals due to the sugar moiety of **5** was performed by means of ^{1}H–^{1}H two dimensional correlation spectroscopy (2D COSY). In the two dimensional nuclear Overhauser effect (NOE) correlation spectroscopy (2D NOESY) spectrum of **5**, the cross peaks were observed between the H-3 of the aglycone moiety and the H-1 of the rhamnoside moiety as well as between the H-3 of the rhamnoside moiety and the H-1 of the glucoside moiety. The anomeric configuration of each sugar unit was elucidated by ^{1}H- and ^{13}C-NMR spectroscopy. Based on these results, **5** was formulated as 3-*O*-β-D-glucopyranosyl(1→3)-α-L-rhamnopyranosyl-(2*R*,3*R*)-taxifolin.

It is well known that dihydroflavonols are readily isomerized even under mild conditions to afford a mixture of stereoisomers arising from asymmetry at 2- and 3-positions. Tominaga has already reported on the formation of **1**, **3** and **4** from **2** by the isomerization reaction with mild alkaline conditions.[4] As shown in Table II, **5** afforded an equilibrium mixture of two isomers, **5** and **7**, but could not detect two other isomers, according to the method of Tominaga.[4] It was revealed that of these conditions, the heating of **5** in 10% pyridine–water was the most optimal condition for the formation of **7**. Compound **7** was isolated by high-performance liquid chromatography (HPLC) from the reaction mixture, and it was found that this isomerized compound, **7**, also tastes sweet. In the ^{1}H-NMR spectrum of **7**, the signals assignable to the H-2 and H-3 of the aglycone moiety appeared as doublets with J=11.2 Hz, showing that H-2 and H-3 are in the *trans* relationship, and the signals for H-2 of aglycone and H-6 of the rhamnosyl moiety are shifted upfield from those of **5**. On going from **5** to **7**, the ^{13}C-NMR signals due to C-3 and -4 were shifted, while other signals remained almost unshifted. Further, **7** exhibited a CD curve which is



	R
1: (2*S*,3*S*)	-Rha
2: (2*R*,3*R*)	-Rha
3: (2*R*,3*S*)	-Rha
4: (2*S*,3*R*)	-Rha
5: (2*R*,3*R*)	-Rha^3–Glc
6: (2*R*,3*R*)	-H
7: (2*S*,3*S*)	-Rha^3–Glc

Rha: α-L-rhamnopyranosyl
Glc: β-D-glucopyranosyl

Chart 1

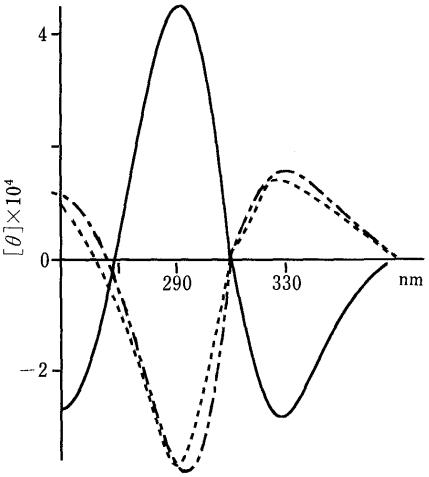


Fig. 1. CD Spectra of **2**, **5** and **7** in MeOH
——, **7**; – – –, **5**; – – –, **2**.

TABLE I. ^{13}C Chemical Shifts of **5**, **6** and **7** (in Acetone-d_6+D_2O)

Carbon No.	5	7	6
Aglycone moiety			
2	83.2	83.0	84.1
3	78.1	75.7	72.7
4	195.7	197.5	197.7
5	164.7	164.8	164.2
6	97.1	97.2	97.1
7	168.1	168.3	167.8
8	96.1	96.1	96.1
9	163.6	163.8	163.6
10	102.0	101.7	101.3
1′	128.6	129.4	129.1
2′	115.4	115.5	116.1
3′	145.8	146.1	145.4
4′	146.7	146.9	146.3
5′	116.3	116.1	115.8
6′	120.0	120.3	120.7
Rhamnosyl moiety			
1	101.5	101.9	
2	70.2[a]	70.6	
3	82.5	82.2	
4	70.4[a]	70.6	
5	69.8	69.5	
6	18.0	18.0	
Glucosyl moiety			
1	105.0	105.0	
2	74.6	74.9	
3	76.8[b]	77.0[a]	
4	71.6	71.5	
5	77.0[b]	77.3[a]	
6	61.7	62.0	

a, *b*) These assignments may be interchanged in each column.

TABLE II. Isomerization of **5**

Condition	5	7
10% pyridine–H_2O[a]	44.0%	56.0%
1% AcONa–EtOH[b]	97.8%	2.2%
H_2O[c]	95.0%	5.0%

a) See experimental. *b*) At room temperature. *c*) Heated at 90 °C.

antipodal to that of **5** as shown in Fig. 1. Based on these results, **7** can be formulated as 3-*O*-*β*-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(2*S*,3*S*)-taxifolin. The name neohuangqioside E is proposed for **7**.

It has already been observed[1] that in the case of 3-*O*-α-rhamnosides of taxifolin, only the glycoside of the (2*S*,3*S*)-isomer (neoastilbin) is sweet and those of other stereoisomers are all tasteless. It is noteworthy that in the case of 3-*O*-(glucosyl-rhamnosides) of the present study, glycosides of both (2*S*,3*S*)- and (2*R*,3*R*)-isomers are found to taste sweet.

Experimental

The melting point was determined on a Yanaco micro hot stage and is uncorrected. NMR spectra were recorded on a JEOL JNM GX-400 instrument using tetramethylsilane (TMS) as an internal standard. Optical rotations were measured with a Union PM-101 automatic digital polarimeter. CD curves were taken on a JASCO J-40A spectropolarimeter. For gas liquid chromatography (GLC), a Shimadzu GC-8A apparatus was used. HPLC was carried out on a column of TSK-gel ODS-120T (7.8 mm × 30 cm) with a Tosoh HLC 803D pump and a Tosoh UV-8 model II spectrophotometer as a detector.

Extraction and Separation of 5 The leaves of *Engelhardtia chrysolepis* (1 kg) which were collected at Gaoyiao-Xian, Guangdon, China, and dried at room temperature, were extracted with hot MeOH. The MeOH extract (253 g) was defatted with Et_2O and chromatographed on a highly porous synthetic polymer (Diaion HP-20, Mitsubishi Chem. Ind. Co., Ltd) with H_2O, 50% MeOH, MeOH and Me_2CO, successively. The fraction eluted with 50% MeOH (93 g) was dissolved in H_2O, and insoluble substances were filtered off. The H_2O-soluble fraction (53.7 g) was chromatographed on a column of silica gel with $CHCl_3$–MeOH–H_2O (80:16:1, homogeneous), affording eight fractions (frs. 1—8), in increasing order of polarity. Fraction 6 was further chromatographed on a column of silica gel with $CHCl_3$–MeOH–H_2O (30:10:1, homogeneous) and then purified by HPLC [mobile phase, 20% CH_3CN–H_2O (containing 0.05% of trifluoroacetic acid, TFA); flow rate, 6 ml/min; detection, UV 254 nm], affording **5** in a yield of 0.036% from dried leaves.

Compound **5**: Colorless needles (from MeOH–H_2O), mp 229—231 °C, $[\alpha]_D^{25}$ −20° (*c*=0.2, EtOH). *Anal.* Calcd for $C_{27}H_{32}O_{16}\cdot 2H_2O$: C, 50.0; H, 5.60. Found: C, 50.26; H, 5.32. ^1H-NMR (acetone-d_6–D_2O) δ: 1.19 (3H, d, *J*=6.2 Hz, Rha H-6), 3.32 (1H, dd, *J*=7.7, 9.4 Hz, Glc H-2), 3.43 (1H, m, Glc H-5), 3.44 (1H, m, Glc H-4), 3.51 (1H, dd, *J*=9.4, 9.8 Hz, Glc H-3), 3.54 (1H, dd, *J*=9.5, 9.6 Hz, Rha H-4), 3.74 (1H, dd, *J*=4.2, 12.1 Hz, Glc H-6), 3.77 (1H, dd, *J*=3.3, 9.5 Hz, Rha H-3), 3.84 (1H, dd, *J*=1.7, 3.3 Hz, Rha H-2), 3.89 (1H, dd, *J*=2.0, 12.1 Hz, Glc H-6), 4.14 (1H, d, *J*=1.7 Hz, Rha H-1), 4.24 (1H, m, Rha H-5), 4.52 (1H, d, *J*=7.7 Hz, Glc H-1), 4.64 (1H, d, *J*=10.6 Hz, H-3), 5.16 (1H, d, *J*=10.6 Hz, H-2), 5.99 (1H, d, *J*=2.0 Hz, H-8), 6.01 (1H, d, *J*=2.0 Hz, H-6), 6.93 (1H, br s, H-5′), 6.93 (1H, br s, H-6′), 7.06 (1H, s, H-2′). ^{13}C-NMR data are given in Table I.

Acid hydrolysis of **5** followed by identification of the resulting monosaccharides were carried out as described in the previous paper.[2]

Isomerization of 5 and Isolation of 7 A solution of **5** (59 mg) in C_5H_5N–H_2O (1:9, 10 ml) was heated at 75 °C for 3.5 h, according to the method reported by Tominaga.[4] The equilibrium mixture was diluted with H_2O and then extracted with *n*-BuOH. The BuOH layer was concentrated to dryness. The residue was purified by HPLC (mobile phase, 20% CH_3CN–0.05% TFA; flow rate, 6 ml/min; detection, UV 254 nm) to give **7** (4.2 mg).

Compound **7**: A white powder, $[\alpha]_D^{18}$ −103° (*c*=0.21, EtOH). HR-FAB-MS *m/z* $(M+H)^+$: Calcd for $C_{27}H_{33}O_{16}$ 613.1769. Found: 613.1758. ^1H-NMR (acetone-d_6–D_2O) δ: 0.91 (3H, d, *J*=6.3 Hz, Rha H-6), 4.13 (1H, d, *J*=1.5 Hz, Rha-1), 4.54 (1H, d, *J*=7.9 Hz, Glc H-1), 4.70 (1H, d, *J*=11.2 Hz, H-3), 5.09 (1H, d, *J*=11.2 Hz, H-2), 5.97 (1H, d, *J*=2.0 Hz, H-8), 6.01 (1H, d, *J*=2.0 Hz, H-6), 6.91 (1H, br s, H-5′), 6.91 (1H, br s, H-6′), 7.07 (1H, s, H-2′). ^{13}C-NMR data are given in Table I.

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