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Sweet Dihydroflavonol Rhamnoside from Leaves of *Engelhardtia chrysolepis*, a Chinese Folk Medicine, Hung-qi

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From leaves of *Engelhardtia chrysolepis*, a Chinese sweet tea, a set of the diastereomers of astilbin (3-*O*-α-L-rhamonosyl-(2*R*,3*R*)-taxifolin) was isolated together with eucryphin and quercitrin. Of these isomers, 3-*O*-α-L-rhamnosyl-(2*S*,3*S*)-taxifolin (neoastilbin) was found to taste sweet. This is the first discovery of the sweetness of a dihydroflavonol glycoside.

Keywords——*Engelhardtia chrysolepis*; Juglandaceae; Chinese folk medicine; dihydroflavonol glycoside; sweet glycoside; astilbin; neoastilbin; taxifolin rhamnoside; huang-qi

A subtropical tree, *Engelhardtia chrysolepis* HANCE (Chinese name: 黄杞, huang-qi; Juglandaceae) grows wild in Guangdong, Guangxi and Fujian, China. Leaves of this plant (a Chinese folk medicine) taste sweet and have been used as a sweet tea.

A methanolic extract of the leaves, which were collected at Gaoyiao-Xian, Guangdon, and dried at room temperature, was washed with ether and then subjected to repeated chromatography to give six compounds, **1**—**6**. Of these compounds, only **4** tastes sweet. Compound **1** was identified as eucryphin which has already been isolated from *Eucryphia coardifolia* CAV. (Eucryphiaceae).[1] Compound **2** was identical with quercitrin, the common quercetin α-L-rhamnoside.

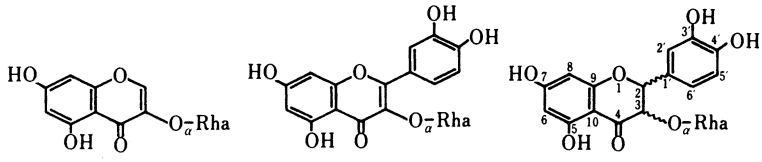
Compound **3** (yield: 0.65%), $[\alpha]_D^{25}$ −14.6° (c=0.52, EtOH) afforded L-rhamnose on acid hydrolysis. Hydrolysis of **3** with crude hesperidinase yielded an aglycone (**7**) which was identified as the known dihydroflavonol, (+)-taxifolin (2*R*,3*R*) by comparison of the optical rotation ($[\alpha]_D^{18}$ +21.1° (c=0.56, EtOH), +24.6° (c=0.57, acetone)) and the ^{1}H- and ^{13}C-nuclear magnetic resonance (^{1}H- and ^{13}C- NMR) spectra with those of an authentic sample. On going from **7** to **3**, glycosylation shifts were observed at the ^{13}C-NMR signals due to C-2, -3, -4 and -1′, while other signals remained almost unshifted.[2] The carbon signals due to the sugar moiety of **3** revealed the presence of one α-rhamnoside unit.[3] Based on these results, **3** was proved to be identical with the known 3-*O*-α-L-rhamnosyl-(2*R*,3*R*)-taxifolin named astilbin.[4,5]

Compound **4** (yield: 0.01%), $[\alpha]_D^{23}$ −71.1° (c=0.55, EtOH), tastes sweet. The ^{1}H-NMR spectrum of **4** differed considerably from that of **3** only with respect to the signal due to a methyl group of the rhamnosyl moiety and the carbon signals of **4** other than those due to C-2 and -3 appeared at almost the same positions as those of **3**. Further, **4** exhibited a circular dichroism (CD) curve which is antipodal to that of **3**. Based on these results, **4** can be formulated as 3-*O*-α-L-rhamnosyl-(2*S*,3*S*)-taxifolin, which has been obtained from **3** by heating with aqueous pyridine or ethanolic sodium acetate, being named neoastilbin by Tominaga.[6] This is the first report of the sweetness of a dihydroflavonol glycoside, though very recently, Kinghorn mentioned the sweetness of 3-*O*-acetyl-(2*R*,3*R*)-taxifolin in a review

article.[7] Preparation of **4** from **3** in a sufficient quantity testing of the relative sweetness with respect to sucrose is under way.

Compound **5** (yield: 0.06%) $[\alpha]_D^{18}$ $-196°$ ($c=0.27$, EtOH) and compound **6** (yield: 0.003%), $[\alpha]_D^{20}$ $+50.7°$ ($c=0.55$, EtOH) afforded L-rhamnose on acid hydrolysis. Inspection of the ^{1}H- and ^{13}C-NMR spectra and the CD-curves of **5** and **6** led to the formulation as 3-*O*-α-L-rhamnosyl-(2*S*,3*R*)-taxifolin (isoastilbin) and 3-*O*-α-L-rhamnosyl-(2*R*,3*S*)-taxifolin (neoisoastilbin), respectively, both of which have also been obtained from **3** together with **4** by Tominaga.[6]

Although to the best of our knowledge, the present study is the first example of the isolation of a set of diastereomers of **3** from a natural source, **4**—**6** might be formed from **3** during the process of extraction or separation. It has been mentioned in China that the sweetness of the leaves of this plant increases on heating so that comparison of the content of **4** in the heated leaves with that in the leaves dried without heating would be desirable.



1 2 3 : 2*R*, 3*R*; 4 : 2*S*, 3*S*; 5 : 2*S*, 3*R*; 6 : 2*R*, 3*S*

Chart 1

Experimental

General Procedure——Melting points were determined on a Yanaco micro hot stage and are uncorrected. Optical rotations were measured with a Union PM-101 automatic digital polarimeter. CD curves were taken on JASCO J-20 and JASCO J-40A spectropolarimeters. NMR spectra were recorded on a JEOL FX-100 instrument using tetramethylsilane (TMS) as an internal standard. For gas liquid chromatography (GLC), a Shimadzu GC-6A apparatus was used. High-performance liquid chromatography (HPLC) was carried out on a column of TSK-gel ODS-120T (21.5 mm × 30 cm) with a Toyo Soda HLC 803D pump and a Toyo Soda RI-8 differential refractometer as a detector.

Acid hydrolysis of glycosides followed by identification of the resulting monosaccharides were carried out as described in the previous paper.[8]

Extraction and Separation of Glycosides——The leaves of *Engelhardtia chrysolepis* (1 kg), collected at Gaoyiao-Xian, Guangdon, China, and dried at room temperature, were extracted with MeOH. The MeOH extract (253 g) was defatted with Et_2O and chromatographed on a column of highly porous 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. The separation was monitored by thin layer chromatography (TLC) on silica gel [solvent: $CHCl_3$–MeOH–H_2O (6 : 4 : 1, homogeneous), detection: 10% H_2SO_4, heating)]. Fraction 2 was crystallized from MeOH to give **1** in a yield of 0.13%. Fraction 4 was purified by preparative HPLC with 20% MeCN in 0.05 M NaH_2PO_4 to give **2**—**6** in yields of 0.006, 0.65, 0.009, 0.003 and 0.055%, respectively. Compounds **1** and **2** were identified as eucryphin and quercitrin, respectively, by comparison of the melting point, $[\alpha]_D$ and NMR data with reference data.[1]

Compound **3**: Colorless needles (from MeOH–H_2O). mp 190—192 °C. $[\alpha]_D^{25}$ $-14.6°$ ($c=0.52$, EtOH). CD ($c=0.0000222$, MeOH) $[\theta]^{26}$ (nm): 0 (266), -4.9×10^4 (293) (negative maximum), 0 (310), $+1.4\times10^4$ (326) (positive maximum), 0 (400). ^{1}H-NMR (acetone-d_6–D_2O) δ: 1.23 (3H, d, $J=6$ Hz, Rha-H-6), 4.66 (1H, d, $J=10$ Hz, H-3), 5.20 (1H, d, $J=10$ Hz, H-2), 6.02 (1H, br s, H-6), 6.05 (1H, br s, H-8), 6.95 (2H, br s, H-5′, 6′), 7.10 (1H, br s, H-2′).

Compound **4**: A white powder $[\alpha]_D^{23}$ $-71.1°$ ($c=0.55$, EtOH). CD ($c=0.0000244$, MeOH) $[\theta]^{26}$ (nm): 0 (275), 4.1×10^4 (292) (positive maximum), 0 (312), -1.6×10^4 (332) (negative maximum), 0 (400). ^{1}H-NMR (acetone-d_6–D_2O) δ: 0.91 (3H, d, $J=6$ Hz, Rha-H-6), 4.71 (1H, d, $J=11$ Hz, H-3), 5.08 (1H, d, $J=11$ Hz, H-2), 5.19 (1H, d, $J=$

TABLE I. ^{13}C-NMR Chemical Shifts of Compounds **3**—**7** in Acetone-d_6–D_2O

Carbon	7[a]	7	3	4	5	6
C-2	84.5	84.1	82.8	83.0	81.4	81.4
C-3	73.1	72.7	77.6	76.0	77.1	74.6
C-4	198.1	197.7	195.2	197.3	193.2	193.9
C-5	165.0	164.2	164.3	164.7	164.9	165.2
C-6	97.1	97.1	97.2	97.2	97.2	97.2
C-7	167.9	167.8	167.8	168.3	168.8	168.1
C-8	96.1	96.1	96.1	96.1	96.2	96.2
C-9	164.1	163.6	163.2	163.7	163.6	163.8
C-10	102.0	101.3	101.9	101.6	101.3	101.3
C-1′	129.8	129.1	128.4	129.4	127.8	127.9
C-2′	115.8	115.8[b]	116.4[b]	116.1[b]	116.1[b]	116.2[b]
C-3′	145.7	145.4	145.6	146.0	145.4	145.7
C-4′	146.6	146.3	146.4	146.8	146.0	146.0
C-5′	115.8	116.1[b]	115.4[b]	115.4[b]	115.3[b]	115.0[b]
C-6′	120.8	120.7	120.2	120.5	119.5	119.2
Rhamnose						
C-1			101.2	102.0	101.6	99.5
C-2			70.8[c]	71.3[c]	70.7[c]	71.2[c]
C-3			71.4[c]	71.5[c]	71.5[c]	71.4[c]
C-4			72.8	73.0	72.7	72.5
C-5			70.0[c]	69.7[c]	70.0[c]	69.8[c]
C-6			17.7	17.8	17.4	17.6

a) Reference data reported by Nishioka *et al.*[9] *b*, *c*) These assignments may be interchanged in each column.

2 Hz, Rha-H-1), 5.97 (1H, d, J=2 Hz, H-6), 6.01 (1H, d, J=2 Hz, H-8), 6.90 (2H, br s, H-5′, 6′), 7.09 (1H, br s, H-2′).

Compound **5**: A white powder. $[\alpha]_D^{18}$ −196° (c=0.27, EtOH). CD (c=0.000022, MeOH) $[\theta]^{25}$ (nm): 0 (265); $+4.9\times10^4$ (295) (positive maximum), 0 (327), -1.3×10^4 (341) (negative maximum), 0 (370). ^{1}H-NMR (acetone-d_6–D_2O) δ: 1.16 (3H, d, J=6 Hz, Rha-H-6), 4.20 (1H, d, J=2 Hz, H-3), 5.44 (1H, d, J=2 Hz, H-2), 6.04 (2H, br s, H-6, 8), 6.93 (2H, br s, H-5′, 6′), 7.13 (1H, br s, H-2′).

Compound **6**: A white powder. $[\alpha]_D^{20}$ +50.7° (c=0.55, EtOH). CD (c=0.0000227, MeOH) $[\theta]^{25}$ (nm): 0 (265), -4.1×10^4 (299) (negative maximum), 0 (327), $+1.4\times10^4$ (341) (positive maximum), 0 (375). ^{1}H-NMR (acetone-d_6–D_2O) δ: 0.97 (3H, d, J=6 Hz, Rha-H-6), 4.30 (1H, d, J=2 Hz, H-3), 5.00 (1H, d, J=2 Hz, Rha-H-1), 5.54 (1H, d, J=2 Hz, H-2), 6.02 (1H, d, J=2 Hz, H-6), 6.07 (1H, d, J=2 Hz, H-8), 6.91 (1H, br s, H-5′ or 6′), 6.92 (1H, br s, H-6′ or 5′), 7.07 (1H, br s, H-2′). ^{13}C-NMR data for **3**—**6** are presented in Table I.

Enzymatic Hydrolysis of 3——A solution of **3** (133 mg) and crude hesperidinase (133 mg) in H_2O (20 ml) was incubated at 37 °C for 36 h. The reaction mixture was diluted with H_2O and passed through a column of DIAION HP-20 with H_2O and MeOH. The fraction eluted with MeOH was evaporated to dryness to give **7** (37 mg), a white powder, $[\alpha]_D^{18}$ +21.1° (c=0.56, EtOH), +24.6° (c=0.57, acetone), which was identified as (+)-taxifolin by comparison ($[\alpha]_D$, ^{1}H- and ^{13}C-NMR) with an authentic sample.

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