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A novel dimeric flavonol glycoside from *Cynanchum acutum* subsp. *sibiricum*

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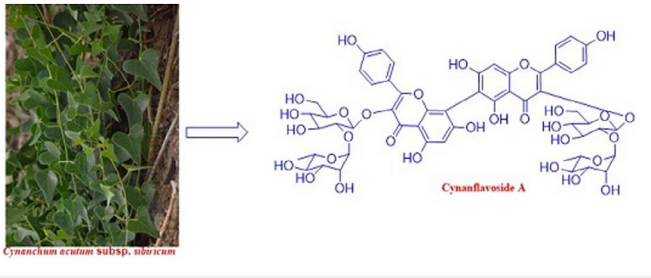
ABSTRACT

A novel dimeric flavonol glycoside, Cynanflavoside A (**1**), together with six analogues, kaempferol-3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranoside (**2**), quercetin-3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranoside (**3**), kaempferol-3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-xylopyranoside (**4**), quercetin-3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-xylopyranoside **(5)**, kaempferol-3-*O*-*β*-D-glucopyranosyl-7-*O*-*α*-L-rhamnopyranoside (**6**), and quercetin-3-*O*-galactoside (**7**) were isolated from the *n*-butyl alcohol extract of *Cynanchum acutum* subsp. *sibiricum*. Their structures were determined spectroscopically and compared with previously reported spectral data. All compounds were evaluated for their anti-complementary activity *in vitro*, and only compound **5** exhibited anti-complement effects with CH_{50} value of 0.33 mM.



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1. Introduction

The genus *Cynanchum* (Asclepiadaceae) includes almost 200 species distributing in east Africa, Mediterranean region, as well as the tropical, subtropical and temperate areas of Europe and Asia (Jiang and Li 1977). There are four species in Xinjiang region of China, one

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of which is *Cynanchum acutum* subsp. *sibiricum* (CASS) (Mijit and Pan 2004). Many herbs of genus *Cynanchum* have been used as antitussive and expectorants in China for a long time and CASS is traditionally used in Uygur medicine for the treatment of dysentery and diarrhea diseases (Mijit and Pan 2004; Pharmacopoeia of the People's Republic of China's 2010). In prior reports, essential oils, flavonoids, alkaloids and C_{21} steroidal glycosides have been isolated from the genus *Cynanchum* (Zhang and Zhou 1983; Yu et al. 2015; Yildiz et al. 2017). About the constituents of CASS, only 4 C_{21} steroidal glycosides and 6 flavonoid glycosides have been reported (Tursunova et al. 1975; Yildiz et al. 2017) and the pharmacological activity of this plant remains unclear. In our search for the anti-complementary agents from traditional medicines, an ethanolic extract of the CASS exhibited anti-complementary activity. Thus, a phytochemical investigation on this plant led to the isolation and characterization of a novel dimeric flavonol glycoside, Cynanflavoside A **(1)**, together with six analogues (**2–7**) from the *n*-butyl alcohol extract of CASS. Additionally, the anti-complementary activity of compound **1–7** was evaluated through the classical pathway.

2. Results and discussion

Compound **1** was obtained as yellow amorphous powder. The HR-ESI-MS gave *m/z* 1211.3039 $[M + Na]^+$, in consistent with the molecular formula $C_{54}H_{56}D_2O_{30}$ (a couple of H protons were irreversibly replaced by D in the Methanol-d_4 after long time placement). Its UV spectrum (λ_{max} 268 and 349 nm) was indicative of its flavonol skeleton. The IR absorption bands at 3145, 1658, 1610, 1505 and 1402 cm^{-1}, indicated the presence of hydroxyl groups, carbonyl groups and aromatic rings in the molecule. The ^1H NMR and ^{13}C NMR spectra (Table S1) showed two sets of A_2B_2-type overlapping doublets, one set at δ_H 8.07(2H, m)/δ_C 132.1, δ_H 6.91(2H, m)/δ_C 116.1, and the other at δ_H 8.06(2H, m)/δ_C 131.9, δ_H 6.89(2H, m)/δ_C 116.0, which are attributed to two AA'BB' systems in two *para*-substituted aromatic rings. The above detailed analysis of the 1D and 2D NMR data, it was possible to conclude that one set belonged to the 2′, 6′ and 3′, 5′ protons of the B (unit I)-ring and the other set to the 2′, 6′ and 3′, 5′ protons of the other B (unit II)-ring. The two singlets at δ_H 6.38 (1H, s)/δ_C 94.7 and δ_H 6.07 (1H, s)/δ_C 99.7 are characteristic of protons H-8 (unit I), and H-6 (unit II) of the two flavones moieties, respectively (The protons of H-8 (unit I) and H-6 (unit II) were irreversibly replaced by D in the Methanol-d_4 after long time placement, and the signals of H-8 (unit I) and H-6 (unit II) were still not appeared when the Methanol-d_4 was replaced by DMSO). The fact that the molecule is non-symmetrical and that the A (unit I) and A (unit II)-ring protons did not show any coupling, meant that the interflavonoid linkage must be of the Spectaflavoside A type (6 (unit I)-8(unit II)) (Sivasothy et al. 2012). These spectral features imply that compound **1** was a dimer of kaempferol, in which the interflavonol linkage was the type 6 (unit I)-8 (unit II) (Ofman et al. 1995; Sivasothy et al. 2012), and both units were very similar to kaempferol-3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside **(2)** (Wu et al. 2009). With the help of the ^1H NMR, ^{13}C NMR and HSQC spectra of compound **1**, we confirmed four anomeric signals of sugars at δ_H 5.82 (H-1″(unit I), 1H, d, J = 7.6 Hz)/δ_C 100.2 (C-1″(unit I)), δ_H 5.24 (H-1‴(unit I), 1H, s)/δ_C 102.9 (C-1‴(unit I)), δ_H 5.76(H-1″ (unit II), 1H, d, J = 7.6 Hz)/δ_C 99.7 (C-1″ (unit II)) and δ_H 5.20 (H-1‴ (unit II), 1H, s)/δ_C 102.6 (C-1‴ (unit II)). The configurations of two β-conformation glucose units were deduced by analysis the coupling constants of the anomeric H-atoms at δ_H 5.82 (1H, d, J = 7.6 Hz), δ_H 5.76 (1H, d, J = 7.6 Hz)

and the ^{1}H and ^{13}C NMR spectra data of compound **1**. Two anomeric proton signals (δ_H 5.24/5.20, each s), two methyl signals (δ_H 0.97/0.95, overlapping), and the ^{1}H and ^{13}C NMR data of compound **1** revealed two α-conformation rhamnose units. The absolute configuration of sugars was confirmed to be D-glucose and L-rhamnose by acid hydrolysis and TLC analysis. The location of two sugar residues were arranged by the HMBC spectrum, in which the anomeric protons δ_H 5.82 (1H, d, $J = 7.6$ Hz, H-1″) and δ_H 5.24 (1H, s, H-1‴) exhibited connectivities with δ_C 134.4 (C-3) and δ_C 80.7 (C-2″), respectively, so the glucose was linked to the flavonoid core at C-3 position, and the rhamnose was linked to the glucose core at C-2″ position in both unit I and II. Consequently, the structure of compound **1** was concluded to be kaempferol-3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside-(I-6,II-8)-kaempferol-3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (named Cynanflavoside A). Dimeric flavonol glycosides constitute a new class of bisflavonol glycosides, and there are only two types, Kunzeagin A and Spectaflavoside A, to be reported till present. Unlike Kunzeagin A in which a methylene bridge connects its two flavonol glycoside monomers, similar to Spectaflavoside A, the two flavonol glycoside monomers in Cynanflavoside A were linked together by a C-C bond (Ito et al. 2004; Sivasothy et al. 2012).

kaempferol-3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside **(2)** (Wu et al. 2009), quercetin-3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside **(3)** (da Silva et al. 2000), kaempferol-3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranoside **(4)** (Ogunlana and Ogunlana 2015), quercetin-3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranoside **(5)** (Kazuma et al. 2003), kaempferol-3-*O*-β-D-glucopyranosyl-7-*O*-α-L-rhamnopyranoside **(6)** (Lee and Der Marderosian 2010) and quercetin-3-*O*-galactoside **(7)** (Ma et al. 2007) were also isolated from the *n*-butyl alcohol extract of CASS. This paper is the first report of compounds **1**, **3**, **5–7** from CASS.

Compounds **1–7** were evaluated for *in vitro* anti-complementary activity, but only compound **5** showed anti-complementary activity with CH_{50} values of 0.33 mM.

3. Experimental

3.1. General

^{1}H NMR (600 MHz, 400 MHz), ^{13}C NMR (150 MHz, 100 MHz) and 2D NMR (HSQC, HMBC) spectra were recorded at room temperature on DRX-600 spectrometer and DRX-400 spectrometer (Bruker, Germany) in CD_3OD and DMSO. UV spectra were measured on a Shimadzu UV-2600 spectrophotometer in absolute MeOH. IR spectra were recorded using an Avatar 360 FT-IR ESP spectrometer (Nicolet, USA) at room temperature. Optical rotations were measured on a JASCO P-1020 polarimeter. HR-ESI-MS spectra were acquired using an Agilent 6210 ESI/TOF mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). L-rhamnose and D-glucose (Alfa Aesar, Great Britain). Analytical TLC were run on silica gel plates (GF_{254}, Yantai Institute of Chemical Technology, Yantai, China). Spots on the plates were observed under UV light and visualized by spraying with 10% H_2SO_4, followed by heating. Column chromatography was performed on silica gel (200~300 mesh and 300~400 mesh; Qingdao Marine Chemical Factory, Qingdao, China), Lichroprep RP_{18} gel (40~60 μm, Merck, Darmstadt, Germany) and reversed-phase MCI gel (CHP20P, Mitsubishi Chemical Inc., Fukuoka, Japan). Distilled solvents were used for chromatographic separations.

3.2. Plant material

The drugs were collected in August 2015 in Shihezi region of Xinjiang Province, P. R. China and identified as *Cynanchum acutum* subsp. *sibiricum* (willd.) Rech.f. by associate professor Peng Li, School of Pharmacy of Shihezi University. The voucher specimens (WQ-CASS-15–1) were deposited in the Herbarium, School of Pharmacy, Shihezi University.

3.3. Extraction and isolation

CASS (aerial parts) was shade dried and powdered. Powdered plant material (14.8 kg) was extracted with 95% EtOH (3 × 20 L, each for 7 d) at room temperature and filtered. The EtOH extract was evaporated in vacuo to give a residue (900 g), a portion of which (850 g) was suspended in H_2O (1.0 L) and partitioned successively with petroleum ether, ethyl acetate, and *n*-butyl alcohol (each 3 × 2 L). The *n*-butyl alcohol extract (178 g) was chromatographyed on a Silica gel (200~300 mesh, 2.5 kg, 14 × 120 cm) column, eluting successively with chloroform-methanol (from 100:0 to 0:100) to yield ten fractions based on TLC profiles. Compound **7** (100 mg) was obtained as a yellow powder from Fraction 2 (10.3 g), which was purified by column chromatography using MCI gel with MeOH-H_2O (from 10:90 to 90:10). Fraction 5 (12.0 g) was subjected to MCI column using MeOH-H_2O (from 10:90 to 60:40) as eluent, followed by repeated column chromatography on RP_{18} gel with MeOH-H_2O (35:65), to give **2** (5 mg), **4** (35 mg) and **6** (6 mg). Fraction 8 (6 g) was subjected to MCI column using MeOH-H_2O (from 10:90 to 50:50) as eluent, followed by repeated column chromatography on RP_{18} gel with MeOH-H_2O (20:80), to yield **1** (2.5 mg), **3** (18 mg) and **5** (20 mg).

3.4. Cynanflavoside A

Yellow amorphous powder; $[\alpha]_D^{22}$: −105 (*c* = 0.02, MeOH); IR (KBr) λ_{max} cm^{-1}: 3145, 1658, 1610, 1505, 1402, 1178, 1066; ^1H NMR (CD_3OD, 600 MHz): Unit I: δ_H 8.07 (2H, m, H-2′, 6′), 6.91 (2H, m, H-3′, 5′), 6.38 (1H, s, H-8), 5.82 (1H, d, *J* = 7.6 Hz, H-1″), 5.24 (1H, s, H-1‴), 4.09 (1H, m, H-5‴), 4.01 (1H, s, H-3‴), 3.76 (1H, m, H-2‴), 3.68 (1H, m, H -6a″), 3.62 (1H, m, H-3″), 3.58 (2H, m, H-2″, 4″), 3.52 (1H, m, H-6b″), 3.37 (1H, m, H-4‴), 3.25 (1H, m, H-5″), 0.97 (3H, m, H-6‴); Unit II: δ_H 8.06 (2H, m, H-2′, 6′), 6.89 (2H, m, H-3′,5′), 6.07 (1H, s, H-6), 5.76 (1H, d, *J* = 7.6 Hz, H-1″), 5.20 (1H, s, H-1‴), 4.07 (1H, m, H-5‴), 3.96 (1H, m, H-3‴), 3.74 (1H, m, H-2‴), 3.63 (1H, m, H-6a″), 3.62 (1H, m, H-3″), 3.57 (2H, m, H-2″, 4″), 3.52 (1H, m, H-6b″), 3.35 (1H, m, H-4‴), 3.25 (1H, m, H-5″), 0.95 (3H, m, H-6‴). ^1H NMR (DMSO-d_6, 600 MHz): Unit I: δ_H 7.97 (2H, m, H-2′, 6′), 6.84 (2H, m, H-3′,5′), 5.70 (1H, m, H-1″), 5.08 (1H, m, H-1‴), 3.73 (1H, m, H-3‴), 3.72 (1H, m, H-5‴), 3.56 (1H, m, H -6a″), 3.47 (2H, m, H-5″, 4‴), 3.41 (1H, m, H-2″), 3.35 (1H, m, H-3″), 3.30 (1H, m, H-6b″), 3.13 (1H, m, H-2‴), 3.09 (1H, m, H-4″), 0.74 (3H, m, H-6‴); Unit II: δ_H 7.97 (2H, m, H-2′, 6′), 6.84 (2H, m, H-3′,5′), 5.67 (1H, d, *J* = 7.6 Hz, H-1″), 5.08 (1H, m, H-1‴), 3.73 (1H, m, H-3‴), 3.72 (1H, m, H-5‴), 3.54 (1H, m, H -6a″), 3.45 (2H, m, H-5″, 4‴), 3.40 (1H, m, H-2″), 3.35 (1H, m, H-3″), 3.30 (1H, m, H-6b″), 3.12 (1H, m, H-2‴), 3.09 (1H, m, H-4″), 0.74 (3H, m, H-6‴) ^{13}C NMR (CD_3OD, 151 MHz): Unit I: δ_C 179.3 (C, C-4), 166.0 (C, C-7), 163.8 (C, C-5), 161.3 (C, C-4′), 158.5 (C, C-9), 158.4 (C, C-2), 134.4 (C, C-3), 132.1 (CH, C-2′, 6′), 123.4 (C, C-1′), 116.1 (CH, C-3′, 5′), 109.8 (C, C-6), 105.8 (C, C-10), 102.9 (CH, C-1‴), 100.2 (CH, C-1″), 94.7 (CH, C-8), 80.7 (CH, C-2″), 79.0 (CH, C-3″), 78.6 (CH, C-5″), 74.1 (CH, C-4‴), 72.4 (CH, C-4″), 72.3 (CH, C-3‴), 71.9 (CH, C-2‴), 69.9 (CH, C-5‴), 62.7 (CH_2, C-6″), 17.5 (CH_3, C-6‴); Unit II: δ_C 178.0 (C, C-4), 165.9 (C, C-7), 163.0

(C, C-5), 161.0 (C, C-4′), 158.3 (C, C-9), 157.2 (C, C-2), 134.1 (C, C-3), 131.9 (CH, C-2′, 6′), 123.1 (C, C-1′), 116.0 (CH, C-3′, 5′), 105.9 (C, C-10), 102.6 (CH, C-1‴), 99.7 (CH, C-6), 99.7 (CH, C-1″), 99.6(C, C-8), 80.1 (CH, C-2″), 78.9 (CH, C-3″), 78.4 (CH, C-5″), 74.0 (CH, C-4‴), 72.3 (CH, C-4″), 72.2 (CH, C-3‴), 71.8 (CH, C-2‴), 69.7 (CH, C-5‴), 62.6 (CH_2, C-6″), 17.5 (CH_3, C-6‴). HR-ESI-MS: *m/z* 1211.3039 $[M + Na]^+$ (Calcd for $C_{54}H_{56}D_2O_{30}Na$ 1211.3031).

3.5. Anti-complementary activity

Assays were based on the degree of hemolysis of erythrocytes after complement activation (Xu et al. 2007). Each tested sample was dissolved in dimethyl sulfoxide (DMSO) and then diluted with Barbital buffer saline (BBS) (pH 7.0, containing 0.1% gelatin, 0.5 mM Mg^{2+} and 0.15 mM Ca^{2+}) to various concentrations. The final concentration of DMSO did not exceed 1% and showed no interference with the anti-complementary activity. Sensitized erythrocytes (EA) were prepared by incubating sheep erythrocytes (4.0 × 108 cells/mL) with rabbit anti-sheep erythrocyte antibody. Various dilutions of tested samples (200 µL) were mixed with 200 µL of diluted guinea pig serum (1:80) and 200 µL EA. The mixture was incubated at 37 °C for 30 min and centrifuged at 1800 g for 5 min. The optical density of the supernatant (200 µL) was measured at 405 nm with a spectrophotometer (Wellscan MK3, Labsystems Dragon). Anti-complementary activity was determined as the mean of triplicate measurements and was expressed as CH_{50}, which corresponded to the concentration that inhibited 50% of hemolysis of the complement-dependent lysis control. Heparin sodium salt was used as the positive control. And the sample controls, the blank control, the complement-dependent lysis control were all prepared under the same conditions.

3.6. Determination of absolute configuration of sugars

The absolute configuration of sugars was carried out by acidic hydrolysis and TLC analysis. Compounds **1** (2.5 mg) was hydrolyzed with concentrated AcOH (3.5 mL), concentrated HCl (1 mL), and H_2O (5.5 mL) at 100 °C for 2 h. The mixture was extracted with EtOAc, and the remaining aqueous phase evaporated to dryness. TLC analysis was performed over silica gel with *n*-butyl alcohol-methanol-chloroform-acetic acid-water (12.5:5:4.5:1.5:1.5, v/v/v/v/v) as mobile phase and the result consistent with authentic samples of D-glucose and L-rhamnose (Figure S10). Compound **1** revealed D-Glc and L-Rha.

4. Conclusion

A novel dimeric flavonol glycoside, Cynanflavoside A **(1)**, together with six known flavonol glycosides were isolated from the *n*-butyl alcohol extract of CASS. Compounds **1**, **3**, **5–7** were isolated from this plant for the first time. The anti-complementary activity of compounds **1–7** was evaluated through the classical pathway and compound **5** showed moderate anti-complementary activity.

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

Chinese Pharmacopoeia Commission. Pharmacopoeia of the People's Republic of China. 2010. Beijing: China Medical Science and Technology Press; p. 1, 101.

Ito H, Kasajima N, Tokuda H, Nishino H, Yoshida T. 2004. Dimeric flavonol glycoside and galloylated C-glucosylchromones from *Kunzea ambigua*. J Nat Prod. 67:411–415.

Jiang Y, Li BT. 1977. Flora of China. Vol. 63. Beijing: Science Press; p. 309.

Kazuma K, Noda N, Suzuki M. 2003. Malonylated flavonol glycosides from the petals of *Clitoria ternatea*. Phytochemistry. 62:229–237.

Lee TM, Der Marderosian AH. 2010. Studies on the constituents of dwarf *gin seng*. Phytother Res. 2:165–169.

Ma XM, Liu Y, Shi YP. 2007. Phenolic derivatives with free-radical-scavenging activities from *Ixeridium gracile* (DC.) Shih. Chem Biodivers. 4:2172–2181.

Mijit H., Pan XL. 2004. Flora Xinjianggensis: Cynanchum L. 4:120–121.

Ofman DJ, Markham KR, Vilain C, Molloy BPJ. 1995. Flavonoid profiles of New Zealand kauri and other species of *Agathis*. Phytochemistry. 38:1223–1228.

Ogunlana OO, Ogunlana OE. 2015. Antiplasmodial flavonoid from young twigs and leaves of *Caesalpinia bonduc* (Linn) Roxb. J Chem Pharm Res. 7:931–937.

da Silva BP, Bernardo RR, Parente JP. 2000. Flavonol glycosides from *Costus spicatus*. Phytochemistry. 53:87–92.

Sivasothy Y, Hadi AHA, Mohamad K, Leong KH, Ibrahim H, Sulaiman SF, Ooi KL, Awang K. 2012. Spectaflavoside A, a new potent iron chelating dimeric flavonol glycoside from the rhizomes of *Zingiber spectabile* Griff. Bioorganic & Medicinal Chemistry Letters. 22:3831–3836.

Tursunova RN, Maslennikova VA, Abubakirov NK. 1975. Pregnane glycosides of *Cynanchum sibiricum* III. The structure of sibiricosides D and E. *Chem Nat Compd*. 11:183–187.

Wu H, Dushenkov S, Ho CT, Sang SM. 2009. Novel acetylated flavonoid glycosides from the leaves of *Allium ursinum*. Food Chem. 115:592–595.

Xu H, Zhang Y, Zhang JW, Chen DF. 2007. Isolation and characterization of an anti-complementary polysaccharide D3-S1 from the roots of *Bupleurum smithii*. Int Immunopharmacol. 7:75–82.

Yildiz I, Sen O, Erenler R, Demirtas I, Behcet L. 2017. Bioactivity-guided isolation of flavonoids from *Cynanchum acutum* L. subsp. *sibiricum* (willd.) Rech. f. and investigation of their antiproliferative activity. Nat Prod Res. 31:2629–2633.

Yu L, Ren JX, Nan HM, Liu BF. 2015. Identification of antibacterial and antioxidant constituents of the essential oils of *Cynanchum chinense and Ligustrum compactum*. Nat Prod Res. 29:1779–1782.

Zhang ZX, Zhou J. 1983. The structures of wallicoside. Acta Chim Sin. 11:1058–1064.