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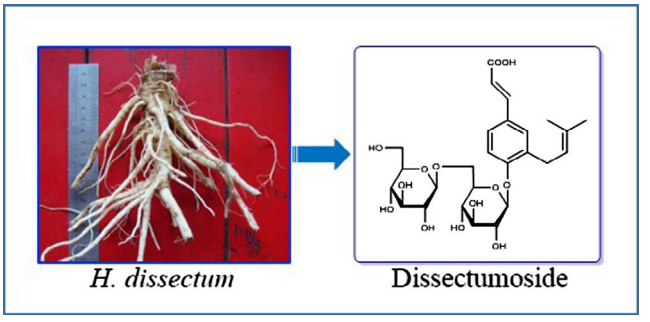
A new cinnamic acid glycoside from roots of *Heracleum dissectum*

Zhi-Gang Wang[a], Jie Mi[b], Xin-Rui Wang[b], Ya-Yu Huo[b], Ya-Jie Peng[b], Hai-Min Zhang[b], Yang Gao[b] and Hai-Long Zhang[b]

[a]Department of Pharmaceutical Analysis, Heilongjiang University of Chinese Medicine, Harbin, China; [b]School of Pharmacy, Xi'an Jiaotong University, Xi'an, China

ABSTRACT

From the roots of *Heracleum dissectum* Lebb., one new cinnamic acid glycoside derivative named dissectumoside (**1**), together with eight known compounds including three phenolics, three phenolic glycosides and two phenylpropanoic glycoside were isolated using various chromatographic methods. Among them compound **2**–**9** was isolated from the plant for the first time. Their structures were elucidated and identified on the basis of their physicochemical properties and by extensive analyses of NMR spectroscopy and high-resolution mass spectrometry. The results of triglyceride accumulation screening in 3T3-L1 cells showed that compounds **1**, **5** and **9** exhibited significantly accelerating activities of adipogenesis in adipocytes.



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Heracleum dissectum; edible Oroqen herb; cinnamic acid glycoside; triglycerides accumulation; 3T3-L1 adipocytes

1. Introduction

Heracleum dissectum is a perennial herb of Apiaceae family, widely distributed in the north-eastern region of China and Far East areas of Russia. Oroqen people, mainly live in North-east China, have eaten the young stems and leaves of *H. dissectum* for a long history not only as the delicious vegetables but also as a remedy for treatment of diabetes and

CONTACT Hai-Long Zhang zhhlcrest@mail.xjtu.edu.cn
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hypertension. Meanwhile, the roots are used as Oroqen folk medicine for dispelling wind and eliminating dampness, curing rheumatoid diseases, waist or knee pain, headache, etc. (Sun and Liu 2007). However, only a few phytochemical studies have been reported on *H. dissectum* in which only essential oils (Papageorgiou et al. 1985; Montanarella et al. 1986) and several simple coumarins (Belenovskaya et al. 1977) were isolated from this edible herb. In our previous study, two new phenylproponoid glycosides were isolated and characterised from the roots (Gao et al. 2014) and the antidiabetic effect of the aerial parts of *H. dissectum* has also been verified in animals (Zhang et al. 2017). Herein, this paper described the isolation and structure elucidation of one new cinnamic acid glycoside (**1**) and eight known compounds (**2**–**9**) and TG accumulation activity in 3T3-L1 cells of all isolates from the roots of *H. dissectum*.

2. Results and discussion

The ethyl acetate fraction and *n*-BuOH fraction of methanolic extract from the dried roots of *H. dissectum* was subjected to column chromatography over silica gel normal-phase, reversed-phase RP-18 and semi-preparative HPLC to give one new cinnamic acid glycoside derivative, drupanin-4-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside, named dissectumoside (**1**) and eight known compounds, isovanillic acid (**2**) (Meng et al. 2000; Zhu et al. 2006), tyrosol (**3**) (Wang et al. 2015; Zhou et al. 2015), catechol (**4**) (Lai et al.1985), praeroside (**5**) (Kong et al.1993; Nagatani et al. 2001), phenethyl-*β*-D-glucopyranoside (**6**) (Xia et al. 2013; Morikawa et al. 2016), tachioside (**7**) (Xiao et al. 2015), isotachioside (**8**) (Liu et al. 2014; Zhou et al. 2016), coniferin (**9**) (Murata et al. 2008; She et al. 2013) (Figure 1).

Compound (**1**) was obtained as a white amorphous powder. The molecular formula was established as $C_{26}H_{36}O_{13}$ by HR–ESI–MS at *m/z*: 579.2070 ([M + Na]$^+$, Calcd 579.2054). In the ^{1}H NMR spectrum, protons at δ_H 7.41 (1H, dd, J = 8.5, 1.9 Hz), 7.31 (1H, d, J = 1.9 Hz) and 7.20 (1H, d, J = 8.5 Hz) revealed that there was a 1, 2, 4-trisubstituted aromatic ring in the structure, and an E-type double bond deduced by the presence of protons at δ_H 7.56 (1H, d, J = 15.9 Hz) and 6.29 (1H, d, J = 15.9 Hz). The ^{13}C NMR and DEPT spectra of **1** showed 26 carbons including one carbonyl carbon, two quaternary aromatic carbons, one oxygenated quaternary aromatic carbon, six olefinic methine carbons, one olefinic quaternary carbon, eight oxymethine carbons, two oxymethylene carbons, two anomeric methine carbons, one methylene carbon and two methyl carbons. In the ^{13}C NMR and HMBC spectra, the carbonyl carbon at δ_C 170.1 was correlated with the protons at δ_H 7.56 (1H, d, J = 15.9 Hz) and 6.29 (1H, d, J = 15.9 Hz), which revealed that the occurrence of a *trans*-cinnamic acid moiety. In addition, carbon at δ_C 133.8 (quaternary carbon) and δ_C 123.4 (its corresponding proton at δ_H 5.32, 1H, t, J = 7.3 Hz) demonstrated the occurrence of a trisubstituted double bond. In the ^{1}H NMR and ^{1}H-^{1}H COSY spectra, protons at δ_H 3.38 (2H, m) correlated with olefinic methine at δ_H 5.32 (1H, t, J = 7.3 Hz). Moreover, HMBC spectrum showed a crossing peaks between two methyl protons at δ_H 1.71 (3H, s) and 1.73 (3H, s) and olefinic quaternary carbon at δ_C 133.8. Above information revealed the presence of prenyl moiety in the molecule which attached on C-3 position of aromatic ring on the basis of observation of crossing peak between proton at δ_H 3.38 (2H, m), 5.32 (1H, t, J = 7.4 Hz) and carbon at δ_C 132.6. The NMR data of **1** was similar to that of drupanin (Schmitt et al. 1991; Hattori et al. 2011), that was 3-prenyl-4-hydroxycinnamic acid.

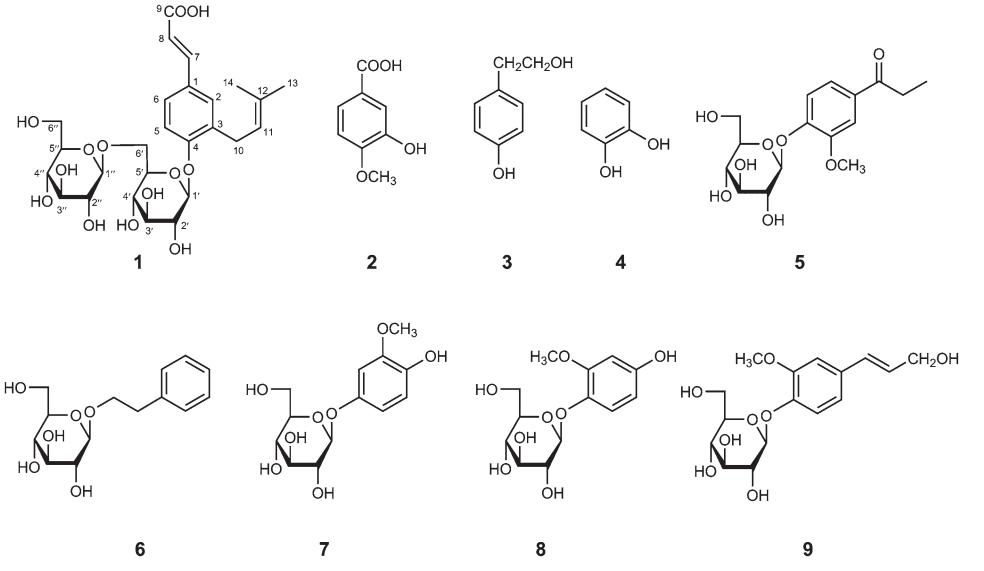

Figure 1. Chemical structures of compounds **1**–**9**.

In the ^{1}H and ^{13}C NMR spectra, two anomeric protons at δ_H 4.94 (1H, d, J = 7.3 Hz) and 4.35 (1H, d, J = 7.7 Hz) and their corresponding carbons at δ_C 101.9 and 104.7 indicated there were two sugar units in the molecule. Acid hydrolysis of compound **1** followed by GC analysis showed the sugar is D-glucose and the configuration of glucose was β-orientation deduce from the coupling constant of their anomeric protons (J = 7.3 and 7.7 Hz, respectively). The outer glucose unit attached to the 6′ position of the inner glucose unit by interpretation of HMBC spectrum, in which proton at δ_H 4.35 (1H, d, J = 7.7 Hz, H-1″) correlated with carbon at δ_C 69.8 (C-6′). Meanwhile, HMBC spectrum of **1** also showed a correlation between proton at δ_H 4.94 (1H, d, J = 7.3 Hz, H-1′) and carbon at δ_C 158.4 (C-4) which indicated that the inner glucose linked to the C-4 position of aromatic ring. Other connections were confirmed by comprehensive analysis of 2D NMR spectroscopic methods, including HMQC, HMBC, ^{1}H–^{1}H COSY and ROESY. Thus, compound **1** was identified as drupanin-4-O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside, and we named compound **1** as dissectumoside.

So far, phytochemical investigation on this plant is few. Only several simple coumarins and essential oil have been isolated and identified from the whole plant of *H. dissectum* (Belenovskaya et al. 1977; Papageorgiou et al. 1985; Montanarella et al. 1986) in the previous study. In this present and our previous study, apart from coumarins, many phenolics and phenylpropenoids including two new lignans and one new cinnamic acid glycoside derivative in this study have been isolated from the aerial parts and the roots of *H. dissectum* for the first time (Gao et al. 2014). These results advance scientific knowledge of chemical constituents of *H. dissectum*.

All compounds **1**–**9** were evaluated for their triglycerides accumulation activity in 3T3-L1 adipocytes (Table 1). Among them, compounds **1**, **5** and **9** showed comparatively potent activity of TG accumulation in cells. Additionally, compounds **6** and **8** also exhibited some accelerating activity at a high concentration (30 μM), while other compounds exhibited weak activity of adipogenesis. In the previous study, the antidiabetic effects of *H. dissectum*

Table 1. Activity of the isolated compounds **1**–**9** from the roots of *H. dissectum* on triglyceride levels in 3T3-L1 adipocytes.

	Increase (%) in TG levels				
Compounds	0	1	3	10	30 (μM)
1	0.0 ± 1.2	7.8 ± 1.2	17.9 ± 2.0**	32.1 ± 2.5**	40.1 ± 2.4**
2	0.0 ± 2.5	1.8 ± 1.0	4.6 ± 1.3	6.2 ± 1.1	5.4 ± 1.6
3	0.0 ± 3.1	−2.0 ± 1.2	−3.2 ± 1.5	4.8 ± 2.1	8.6 ± 2.5
4	0.0 ± 2.4	−4.2 ± 2.4	−9.6 ± 3.8	−12.2 ± 2.5	5.4 ± 3.4
5	0.0 ± 4.0	6.9 ± 1.6	11.5 ± 2.3**	19.8 ± 3.1**	25.4 ± 3.6**
6	0.0 ± 3.4	−1.9 ± 1.1	−6.5 ± 3.4	10.8 ± 2.7	12.1 ± 3.9*
7	0.0 ± 1.3	−3.9 ± 2.8	−8.2 ± 3.0	7.8 ± 2.2	9.4 ± 1.6
8	0.0 ± 4.5	4.7 ± 3.0	6.3 ± 3.3	9.2 ± 1.4	13.4 ± 4.6*
9	0.0 ± 2.8	8.2 ± 3.2	16.3 ± 2.8**	18.2 ± 3.0**	17.6 ± 1.8**
Troglitazone	0.0 ± 1.3	28.6 ± 1.8**	37.2 ± 2.2**	43.6 ± 1.9**	–

Notes: Values represent means ± SEM of % increase in TG levels ($n = 4$). Significantly different from the control group. *$p < 0.05$; **$p < 0.01$.

methnolic extract (HdME) were consolidated *in vivo*, in which HdME could significantly improve glucose tolerance in mice and reduce the elevation of blood glucose in STZ-induced diabetic mice (Zhang et al. 2017). Additionally, the compound exhibiting accelerating activity on TG accumulation *in vitro* might provide antidiabetic effect *in vivo* (Zhang et al. 2007, 2009), because this compound could effectively transport the glucose from the medium into the adipocytes and dispose the glucose into triglyceride. Based on these results, we deduce the new compound, dissectumoside (**1**), might possess antidiabetic effect *in vivo* which needs further evaluation in the future study.

As regards to the structure-activity relationship, as shown in Table 1 and Figure 1, firstly, the activity of glycosides (**1**, **5**, **6**, **7**, **8** and **9**) is better than that of non-glycosides (**2**, **3** and **4**) which demonstrated that glucosyl moiety in molecules is essential for this activity. Secondly, among these glycosides (**1**, **5**, **6**, **7**, **8** and **9**), the activity of compounds (**1**, **5** and **9**) is better than that of others (**6**, **7** and **8**), which reveals that 4-hydroxy phenpropyl moiety in aglycones is important.

3. Experimental

3.1. General experimental procedures

The HR-ESI-MS was run on a Bruker microTOF-Q II mass spectrometer (Bruker Daltonics, Billerica, MA). Nuclear magnetic resonance (NMR) spectra were measured and recorded on a Bruker Avance III-400 spectrometer (Bruker, Rheinstetten, Germany). Tetramethylsilane (TMS) was used as an internal standard. Chemical shifts are reported in δ (ppm), and coupling constants (*J*) are expressed in hertz. Column chromatography (CC) was preformed over silica gel (200–300 mesh, Qingdao Haiyang Chemical Co., Qingdao, China), ODS (100–200 mesh, Fuji Silysia Chemical, Japan) or Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Sweden). Shimadzu semi-preparative HPLC (Shimadzu, Kyoto, Japan), Merges C18 column (250 × 10 mm inner diameter, 5 μm, Hanbon, Suzhou, China) was used for the purification of compounds. Triglyceride (TG) test kits were purchased from Beijing BHKT clinical reagent Co., Ltd (Beijing, China) and their measurements were conducted on a multiplate reader (Synergy HT, BioTek, USA).

3.2. Chemicals

Recombinant insulin, 3-isobutyl-1-methylxanthine (IBMX), dexamethasone and the other chemicals were obtained from Sigma (St. Louis, MO, USA). Foetal calf serum (FCS), penicillin, and streptomycin were obtained from Gibco (Invitrogen).

3.3. Plant material

The roots of *H. dissectum* Ledeb. were collected in September 2015, Tongbei, Heilongjiang Province and authenticated by professor Xiaofeng Niu (The School of Pharmacy, Health Science Center, Xi'an Jiaotong University, Xi'an China). The voucher specimen (KKL 150916) was deposited in the school of Pharmacy, Health Science Center, Xi'an Jiaotong University.

3.4. Extraction and isolation

The air-dried roots of *H. dissectum* (6.5 kg) were extracted three times with methanol (3 × 40 L) under reflux. After filtration, the solvent was evaporated under reduced pressure to yield a crude methanol extract (HdME, 1.02 kg, 15.6%). A part of the residue (560.5 g) was suspended in water (2 L) and successively partitioned with petroleum ether (PE, 4 × 2 L), $CHCl_3$ (4 × 2 L), EtOAc (4 × 2 L) and *n*-BuOH (4 × 2 L) to obtain PE fraction (68.3 g, 12.2%), $CHCl_3$ fraction (94.5 g, 16.8%), EtOAc fraction (9.4 g, 1.7%), *n*-BuOH fraction (130.1 g, 23.2%) and aqueous fraction (253.9 g, 45.3%), respectively.

The EtOAc fraction (9.4 g) was chromatographed on a silica gel column (100–200 mesh) using $CHCl_3$/MeOH (1:0, 50:1, 20:1, 10:1, 5:1, 3:1, 1:1, 0:1, *v/v*) to give 19 fractions FrE1-FrE19 based on TLC analysis.

Fr.E4 (1 g) was divided into two subfractions (FrE4.1–FrE4.2) through a silica gel column (50 g, 2.5 × 50 cm) gradiently eluted with $CHCl_3$/MeOH (100:1, 50:1, 20:1, 1:0, *v/v*) according to the TLC profiles. FrE4.1 was further separated by semi-preparative HPLC with a Megres C18 column (30% MeOH in 1% CH_3COOH with a flow rate of 3.0 mL/min) to give compound **2** (10 mg). FrE4.2 was further chromatographed over semi-preparative HPLC with a Megres C18 column (15% MeOH in 1% CH_3COOH, 3.0 mL/min) to give compound **3** (9 mg) and compound **4** (5 mg).

The *n*-BuOH extract (111.6 g) was separated to silica gel column chromatography and eluted with $CHCl_3$/MeOH (1:0, 100:1, 50:1, 20:1, 10:1, 8:1, 6:1, 3:1, 2:1, 1:1, 0:1, *v/v*). The collected fractions were combined based on their TLC characteristics to afford 30 fractions FrB1-FrB30.

FrB12 (300 mg) was applied to ODS column eluting with MeOH/H_2O (30:70, 50:50, 70:30, 100:0, *v/v*) to give two subfractions FrB12.1–FrB12.2. FrB12.1 was further subjected to semi-preparative HPLC with a Thermo C18 column, which was eluted with 20% MeOH in 0.5% CH_3COOH (3.0 mL/min) to give compound **5** (4 mg). FrB12.2 was purified by semi-preparative HPLC with a Thermo C18 column (25% MeOH in 0.5% CH_3COOH, 3.0 mL/min) to give compound **6** (15 mg).

FrB20 (5 g) was chromatographed on ODS column, successively eluted with MeOH/H_2O (30:70, 45:55, 70:30, 100:0, *v/v*) to yield four subfractions FrB20.1–FrB20.4. FrB20.1 was separated to Sephadex LH-20 column, eluted with MeOH/H_2O (3:7, *v/v*), to obtain two fractions FrB20.1.1–FrB20.1.2. Then FrB20.1.2 was further chromatographed on semi-preparative HPLC

with a Megres C18 column (5% MeOH-H_2O with a flow rate of 3.0 mL/min) to give compound **7** (6 mg) and compound **8** (6 mg). Fr.B20.2 was subjected to Sephadex LH-20 column, eluted with MeOH/H_2O (3:7, *v/v*), to give two fractions FrB20.2.1–FrB20.2.2. And FrB20.2.1 was purified by semi-preparative HPLC with a Megres C18 column using MeOH/H_2O (25:75, *v/v*) as the eluent to give compound **9** (28 mg).

FrB25 (7 g) was applied to ODS column eluting with MeOH/H_2O (40:70, 60:50, 75:30, 100:0, *v/v*) to give three subfractions FrB25.1–FrB25.3. FrB25.2 was further chromatographed on semi-preparative HPLC with a Megres C18 column (45% MeOH in 0.5% CH_3COOH, 3.0 min) to give compound **1** (16 mg).

3.4.1. Drupanin-4-O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (1)

White amorphous powder; IR (KBr) ν_{max}: 3323, 2982, 1650 cm^{-1}; HR-ESI MS at *m/z* 579.2070 $[M + Na]^+$ (Calcd for $C_{26}H_{36}O_{13}$: 579.2054). ^1H NMR (400 MHz, CD_3OD), δ_H: 7.31 (1H, d, *J* = 1.9 Hz, H-2), 7.20 (1H, d, *J* = 8.5 Hz, H-5), 7.41 (1H, dd, *J* = 8.5, 1.9 Hz,H-6), 7.56 (1H, d, *J* = 15.9 Hz, H-7), 6.28 (1H, d, *J* = 15.9 Hz, H-8), 3.38 (2H, m, H-10), 5.32 (1H, t, *J* = 7.4 Hz, H-11), 1.71 (3H, s, H-13), 1.73 (1H, s, H-14), 4.94 (1H, d, *J* = 7.3 Hz, H-1′), 3.48 (1H, m, H-2′), 3.46 (1H, m, H-3′), 3.38 (1H, m, H-4′), 3.69 (1H, m, H-5′), 4.15 (1H, d, *J* = 10.4 Hz, H-6′), 3.80 (1H, m, H-6′), 4.35 (1H, d, *J* = 7.7 Hz, H-1″), 3.19 (1H, m, H-2″), 3.28 (1H, m, H-3″), 3.26 (1H, m, H-4″), 3.17 (1H, m, H-5″), 3.62 (1H, dd, *J* = 12.0, 5.6 Hz, H-6″), 3.81 (1H, m, H-6″); ^{13}C NMR (100 MHz, CD_3OD), δ_C: 129.8 (C-1), 130.1 (C-2), 132.6 (C-3), 158.4 (C-4), 116.3 (C-5), 128.8 (C-6), 146.3 (C-7), 117.3 (C-8), 170.1 (C-9), 29.3 (C-10), 123.4 (C-11), 133.8 (C-12), 18.0 (C-13), 26.0 (C-14), 101.9 (C-1′), 74.9 (C-2′), 78.2 (C-3′), 71.4 (C-4′), 77.6 (C-5′), 69.8 (C-6′), 104.7 (C-1″), 75.5 (C-2″), 77.9 (C-3″), 71.6 (C-4″), 77.9 (C-5″), 62.7 (C-6″).

3.5. Adipogenesis in 3T3-L1 cells

3T3-L1 fibroblasts were seeded into 48-well multiplates (5.0×10^4 cells/well). After incubation for 24 h, the cells were differentiated by changing to a differentiation medium (high glucose DMEM supplemented with 10% FCS, 1 μM dexamethasone, 0.5 mM 3-isobutyl-1-methylxanthine, and 5 μg/mL insulin). After incubation for 3 days, the differentiation medium was replaced by a maintenance medium (high glucose DMEM supplemented with 10% FCS and 5 μg/mL insulin). After another 4 days incubation (the fresh maintenance medium was exchanged every other day), the cells were sonicated in H_2O (200 μL/well). The triglyceride concentration was measured using a commercial kit. The test compounds dissolved in DMSO were added when the medium was changed every time and troglitazone was as a reference compound in this assay.

3.6. Acid hydrolysis of new compound (1)

Compound **1** (3 mg) was heated in 5 mL of 2 M CF_3COOH at 110°C. After 6 h, the reaction mixture was diluted with 15 mL H_2O and extracted with 5 mL EtOAc for three times. The aqueous layer was evaporated, dissolved in anhydrous pyridine (100 μL), cysteine methyl ester hydrochloride (200 μL) was added, and the mixture was warmed at 60°C for 1 h. Then the trimethysilylation reagent HMDS-TMCS (2:1:10) was added and warmed at 60°C for 30 min. The supernatant was subjected to GC for sugar identification. The D-glucose was confirmed by comparison of its retention time (t_R 12.5 min) with that of authentic standard.

4. Conclusion

To conclude, in the course of our continuous search for bioactive components from natural resources, one new cinnamic acid glycoside derivative, drupanin-4-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside (**1**) and eight known compounds (**2**–**9**) have been isolated from the ethyl acetate and *n*-butanol fractions of methanol extract of the roots of *H. disecctum*. This study indicated that phenolic glycosides are the main components of *n*-butanol fraction of *H. disecctum*. Moreover, we investigated the accumulating activity on adipogenesis in 3T3-L1 adipocytes. As a result, the compound **1**, as well as compounds **5** and **9**, exhibited significantly accelerating effects on accumulation of triglycerides in 3T3-L1 cells.

Disclosure statement

No potential conflict of interest was reported by the authors.

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