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FIVE LIGNANS FROM THE MANGROVE *RHIZOPHORA STYLOSA* GRIFF.

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Abstract

Five lignans (–)-(7*R*,8*S*)-dihydrodehydrodiconiferyl alcohol (**1**), (7*S*,8*R*)-3,3′,5-trimethoxy-4′,7-epoxy-8,5′-neolignan-4,9,9′-triol (**2**), (+)-isolariciresinol (**3**), polystachyol (**4**), (+)-pinoresinol (**5**) were isolated from the mangrove plant *Rhizophora stylosa* Griff.. The chemical structures of these compounds were elucidated by analysis of their NMR spectra and compared with those reported references. All these compounds were isolated from this plant for the first time.

Keywords. *Rhizophora stylosa*, mangrove, lignans.

1. INTRODUCTION

Rhizophora stylosa Griff. (Rhizophoraceae) is a common mangrove plant that grows in the coastal areas of Vietnam, from Quang Ninh to Ba Ria – Vung Tau province [1]. Some *Rhizophora* species are used by the local people as folk medicines for many diseases. For example, the bark of *R. mucronata* is used for the treatment of diabetes in India [2], and when boiled in water it is used as an astringent for diarrhea, nausea, and vomiting and as an antiseptic in Thailand [3]. Previous phytochemical studies revealed that flavanols, flavanol glycosides, and triterpenes have been isolated from *R. stylosa.* In addition, bioactivity investigation has shown that some of these compounds displayed antioxidant activity [4, 5, 6]. In this paper, we report the isolation and structural elucidation of five known lignan compounds **1**–**5** from the leaves of this plant.

2. EXPERIMENTAL

2.1. General experimental procedures

NMR spectra were recorded on a Bruker AM500 FT-NMR spectrometer (Bruker, Billerica, MA, U.S.A.) using TMS as an internal standard. The ESI-MS was measured on Agilent 1260 series single quadrupole LC/MS systems. Column chromatography (CC) was performed using a silica gel (Kieselgel 60, 70–230 mesh and 230–400 mesh, Merck, Darmstadt, Germany) or YMC RP-18 resins (30 - 50 μm, Fuji Silysia Chemical Ltd, Aichi, Japan). Thin layer chromatography (TLC) used pre-coated silica gel 60 F_{254} (1.05554.0001, Merck, Darmstadt, Germany) and RP-18 F_{254S} plates (1.15685.0001, Merck, Darmstadt, Germany) and compounds were visualized by spraying with aqueous 10 % H_2SO_4, then heating for 3–5 minutes.

2.2. Plant material

The samples of the mangrove plant *Rhizophora stylosa* Griff. were collected in July 2013 at Xuan Thuy national park, Nam Dinh province, Vietnam and were identified by Dr. Nguyen The Cuong, Institute of Ecology and Biological Resources, VAST. A voucher specimen (No. XT_CB05C) was maintained at the IMBC, VAST.

2.3. Extraction and isolation

Dried leaves of *R. stylosa* (1.8 kg) were powdered and extracted with hot MeOH (three times at 50^{o}C for 6 h each) to give a MeOH residue (200 g, A) after removal of the solvent in vacuum. This extract was suspended in water and partitioned in turn with *n*-hexane and CH_2Cl_2 to provide the corresponding extracts: *n*-hexane (H, 80 g), CH_2Cl_2

(C, 20 g), and a water layer.

Extracts H and C were combined and crudely separated by silica gel CC using a gradient concentration of MeOH in CH_2Cl_2 (0-100 %) to obtain seven fractions (H1–H7). Fraction H4 (4.1 g) was further separated by YMC RP-18 CC and eluted with MeOH–water (2.5:1, v/v), followed by silica gel CC with *n*-hexane–EtOAc (1:1, v/v) to give compound **5** (7.0 mg). Fraction H5 (5.2 g) was subjected to column chromatography on a silica gel column and eluting with CH_2Cl_2/MeOH (15:1, v/v) to afford five subfractions H5A-H-5E. Subfraction H5B was separated by YMC using MeOH/H_2O (1:2) as eluent to give four subfractions H5B1-H5B4. Subfraction H5B1 was purified further by silica gel CC and eluted with CH_2Cl_2/MeOH/H_2O (20:1:0.1) to obtain compound **3** (5.0 mg) and compound **4** (5.5 mg). Separation of subfractions H5B2 through Sephadex LH-20 CC eluted with MeOH/H_2O (1:2) to give compound **1** (4.5 mg) and compound **2** (5.0 mg).

(–)-(7*R*,8*S*)-dihydrodehydrodiconiferyl alcohol (**1**): Yellowish oil; ^{1}H-NMR (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD) see table 1; Positive ESI-MS: *m/z* 361 $[M+H]^+$.

(7*S*,8*R*)-3,3′,5-trimethoxy-4′,7-epoxy-8,5′-neolignan-4,9,9′-triol (**2**): Colorless amorphous solid; ^{1}H-NMR (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD) see table 1. Positive ESI-MS: *m/z* 391 $[M+H]^+$.

(+)-isolariciresinol (**3**): white amorphous powder; ^{1}H-NMR (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD) see table 2. Positive ESI-MS: *m/z* 383 $[M+Na]^+$.

Polystachyol (**4**): white amorphous powder; ^{1}H-NMR (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD) see table 2. Positive ESI-MS: *m/z* 421 $[M+H]^+$.

(+)-pinoresinol (**5**): Colorless oil; ^{1}H-NMR (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD) see table 3. Positive ESI-MS: *m/z* 381 $[M+Na]^+$.

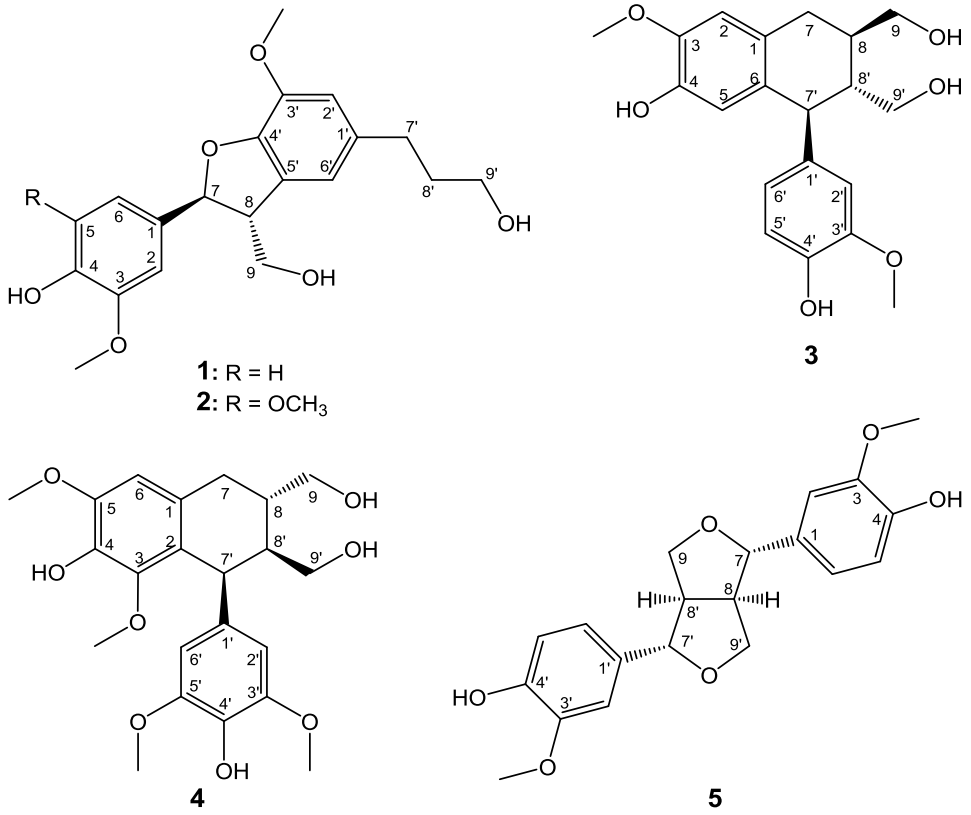

Figure 1: Structures of compounds **1**–**5**

3. RESULTS AND DISCUSSION

Compound **1** was obtained as yellowish oil. The mass spectrometry gave an $[M + H]^+$ ion at *m/z* 361 indicating a mass 360 compatible with the molecular formula of $C_{20}H_{24}O_6$. The 1D and 2D-NMR spectra of **1** revealed that **1** was a derivative of a dihydrobenzofuran-type neolignan. The ^{13}C-NMR and DEPT spectra showed signals for seven aromatic quaternary carbons, four of which are oxy-quaternary; seven methines including five aromatic methines; two oxy-methylenes; two methylenes; and two methoxy carbons. The ^{1}H-NMR spectrum displayed signals for an ABX coupling system at δ

6.97 (1H, d, 2.0, H-2), δ 6.78 (1H, d, 8.0, H-5), δ 6.84 (1H, dd, 2.0, 8.0, H-6) of the 1,3,4-trisubstituted benzene A ring which were connected to a C_7 unit belonging to the signals at δ 5.51 (1H, d, 7.0, H-7), 3.48 (1H, m, H-8), and 3.78 (1H, m, H_a-9)/3.85 (1H, m, H_b-9). The ^{1}H-NMR spectrum also supported the presence of a 1,3,4,5-tetrasubstituted benzen B ring with the signals of a pair of *meta*-coupled at δ 6.75 (1H, br s, H-2′) and 6.75 (1H, br s, H-6′). In the aliphatic region, the signals of two benzylic proton at δ 2.65 (2H, t, 7.5, H-7′), two aliphatic proton at δ 1.84 (2H, m, H-8’) and two oxygenated methylene protons at δ 3.60 (2H, t, 7.0, H-9′) were assigned to a *n*-propanol moiety, that was a C_3 unit of the B ring. Moreover, two singlet signals of two methoxy groups were also observed at δ 3.84 (3H, s, 3-OMe) and 3.87 (3H, s, 3′-OMe). In the HMBC spectrum, the correlations (Fig. 2) from H-7 to C-1, C-2, C-6, C-8, C-9, C-4′, C-5′ and from H-8 to C-9, C-7, C-1, C-4′, C-5′ indicated that dihydrobenzofuran moiety was attached to C-1 of the aromatic A ring. Similarly, the HMBC cross peaks between H-7′ and C-1′, C-2′, C-6′, C-8′, C-9′ revealed the *n*-propanol moiety was linked to C-1′ of the aromatic B ring. The coupling constant (J = 7.0 Hz) between H-7 and H-8 indicated the relative *trans*-vicinal coupling of 7-aryl, 8-hydroxymethyl to the dihydrobenzofuran ring. On the basis of the above evidence, the structure of **1** was identified as (−)-(7*R*,8*S*)-dihydrodehydrodiconiferyl alcohol by comparison of spectral data with those reported in the literature [7].

Compound **2**, a colorless amorphous solid, exhibited a molecular ion peak $[M + H]^+$ at *m/z* 391 in ESI-MS, suggesting a molecular formula of $C_{21}H_{26}O_7$. The data of ^{1}H- and ^{13}C-NMR spectra of **2** were very similar to those of compound **1** except for the appearance of a methoxy group at C-5 position. So, the ^{1}H-NMR spectrum of **2** showed the presence of an AB coupling system in the aromatic A ring, represented by a pairs of broad singlet at δ 6.70 (2H, br s, H-2, 6). Moreover, the HMBC correlations were observed from proton of 5-OMe to C-5 indicating that methoxy group attached at the C-5. Thus, **2** was identified to be (7*S*,8*R*)-3,3′,5-trimethoxy-4′,7-epoxy-8,5′-neolignan-4,9,9′-triol by comparison with the reported chemical data [8].

Table 1: The NMR data of compounds **1** and **2**

C	1			2		
	$^{\#}\delta_C$	δ_C [a, b]	δ_H [a, c] (J = Hz)	$^{\#\#}\delta_C$	δ_C [a, b]	δ_H [a, c] (J = Hz)
1	134.8	134.83	-	132.2	134.06	-
2	110.5	110.57	6.97 (1H, d, 2.0)	103.1	104.17	6.70 (1H, br s)
3	149.1	149.10	-	147.1	147.34	-
4	147.5	147.50	-	134.6	136.36	-
5	116.1	116.14	6.78 (1H, d, 8.0)	147.1	147.34	-
6	119.7	119.71	6.84 (1H, dd, 2.0, 8.0)	103.1	104.17	6.70 (1H, br s)
7	89.0	88.99	5.51 (1H, d, 7.0)	88.1	89.10	5.52 (1H, d, 6.5)
8	55.4	55.45	3.48 (1H, m)	53.8	55.56	3.49 (1H, m)
9	65.0	65.78	3.78 (1H, m) 3.85 (1H, m)	63.7	65.00	3.78 (1H, m) 3.85 (1H, m)
3-OMe	56.4	56.39	3.84 (3H, s)	56.3	56.78	3,83 (3H, s)
5-OMe	-	-	-	56.3	56.78	3,83 (3H, s)
1′	136.9	136.92	-	135.5	137.01	-
2′	115.8	114.15	6.75 (1H, br s)	112.4	114.16	6.75 (1H, br s)
3′	145.2	145.21	-	144.2	146.20	-
4′	147.5	147.55	-	146.5	147.50	-
5′	129.9	129.91	-	127.7	129.81	-
6′	117.9	117.94	6.75 (1H, br s)	115.9	117.94	6.75 (1H, br s)
7′	32.9	32.91	2.65 (2H, t, 7.5)	32.0	32.88	2.65 (2H, t, 7.5)
8′	35.8	35.81	1.84 (2H, m)	34.6	35.77	1.84 (2H, m)
9′	62.2	62.24	3.60 (2H, t, 7.0)	62.2	62.23	3.59 (2H, t, 7.0)
3′-OMe	56.7	56.78	3.87 (3H, s)	56.0	56.81	3.88 (3H, s)

[a]recored in CD_3OD, [b]125 MHz, [c]500 MHz, $^{\#}\delta_C$ of (−)-(7*R*,8*S*)-dihydrodehydrodiconiferyl alcohol in CD_3OD [7], $^{\#\#}\delta_C$ of (7*S*,8*R*)-3,3′,5-trimethoxy-4′,7-epoxy-8,5′-neolignan-4,9,9′-triol in $CDCl_3$ [8].

Compound **3** was isolated as a white amorphous powder. A molecular formula of $C_{20}H_{24}O_6$ was determined for compound **3** on the basis of the observation of a molecular ion peak $[M+Na]^+$ at *m/z* 383 in ESI-MS. The ^{13}C-NMR and DEPT spectroscopic data showed signals for seven aromatic quaternary carbons, four of which are oxy-quaternary; eight methines including five aromatic methines; one methylene; two oxy-methylenes and two methoxy carbons. The ^{1}H-NMR spectrum displayed signals for a 1,3,4-trisubstituted aromatic ring at δ 6.70 (1H, d, 2.0, H-2′), δ 6.76 (1H, d, 8.0, H-5′), δ 6.63 (1H, dd, 2.0, 8.0, H-6′), and signals for a 1,3,4,5-tetrasubstituted benzene ring at δ 6.68 (1H, s) and 6.21 (1H, s). It further showed two methoxy groups at δ 3.83 (3H, s, 3-OMe), δ 3.80 (3H, s, 3′-OMe), two oxygenated methylene group with overlapped signals at δ 3.69 (1H, H_a-9)/3.72 (1H, H_b-9), δ 3.70 (1H, H_a-9′)/3.42 (1H, dd, 4.0, 11.0, H_b-9′), a methylene group at δ 2.79 (2H, d, 7.5, H-7), three methine group at δ 3.82 (1H, H-7’), 2.02 (1H, m, H-8), 1.80 (1H, m, H-8′). The ^{1}H- and ^{13}C-NMR spectra strongly implied that compound **3** could be a lignan. In the HMBC spectrum, the correlations between H-7 and C-1, C-2, C-6, C-8, C-9, C-8′, and between H-7′ and C-1, C-2, C-6, C-8, C-1′, C-2′, C-6′, C-8′, C-9′ revealed **3** to be an aryl-tetralin type lignan. The HMBC correlation (Fig. 2) from proton 3-OMe to C-3 and from proton 3′-OMe to C-3′ confirmed the positions of two methoxy group. The absolute configuration of **3** was determined on the basis of the good agreement of NMR spectral data with those of (+)-isolariciresinol [9]. Therefore, compound **3** was identified to be (+)-isolariciresinol.

Table 2: The NMR data of compounds **3** and **4**

C	3			4		
	$^{\#}\delta_C$	δ_C [a, b]	δ_H [a, c] (*J* = Hz)	$^{\#\#}\delta_C$	δ_C [a, b]	δ_H [a, c] (*J* = Hz)
1	129.0	129.04	-	130.2	130.20	-
2	112.4	112.41	6.68 (1H, s)	126.2	126.25	-
3	147.2	147.22	-	147.7	147.70	-
4	145.3	145.29	-	138.9	138.89	-
5	117.3	117.36	6.21 (1H, s)	148.7	148.67	-
6	134.2	134.18	-	107.8	107.81	6.61 (1H, s)
7	33.6	33.57	2.79 (2H, d, 7.5)	33.6	33.55	2.59 (1H, dd, 11.5, 15.0) 2.72 (1H, dd, 5.0, 15.0)
8	40.0	40.01	2.02 (1H, m)	40.9	40.89	1.65 (1H, m)
9	65.9	65.97	3.69[d] (1H) 3.72[d] (1H)	66.8	66.80	3.51[d] (1H) 3.62 (1H, dd, 5.0, 12.0)
1′	138.6	138.63	-	139.3	139.31	-
2′	113.8	113.83	6.70 (1H, d, 2.0)	106.8	106.90	6.40 br (1H, s)
3′	149.0	149.03	-	149.0	148.99	-
4′	145.9	145.95	-	134.5	134.54	-
5′	115.9	115.99	6.76 (1H, d, 8.0)	149.0	148.99	-
6′	123.2	123.11	6.63 (1H, dd, 2.0, 8.0)	106.8	106.90	6.40 (1H, s)
7′	48.1	48.05	3.82[d] (1H)	42.3	42.29	4.33 (1H, d, 5.5)
8′	48.0	48.02	1.80 (1H, m)	49.6	49.50	2.00 (1H, m)
9′	62.2	62.25	3.70[d] (1H) 3.42 (1H, dd, 4.0, 11.0)	64.1	64.20	3.51[d] (2H)
3-OMe	56.4	56.41	3.83 (3H, s)	60.1	60.17	3.40 (3H, s)
5-OMe	-	-	-	56.6	56.62	3.88 (3H, s)
3′-OMe	56.3	56.37	3.80 (3H, s)	56.7	56.78	3.76 (3H, s)
5′-OMe	-	-	-	56.7	56.78	3.76 (3H, s)

[a]recored in CD_3OD, [b]125 MHz, [c]500 MHz, [d]overlapped signal, $^{\#}\delta_C$ of (+)-isolariciresinol in CD_3OD [9], $^{\#\#}\delta_C$ of polystachyol in CD_3OD [10].

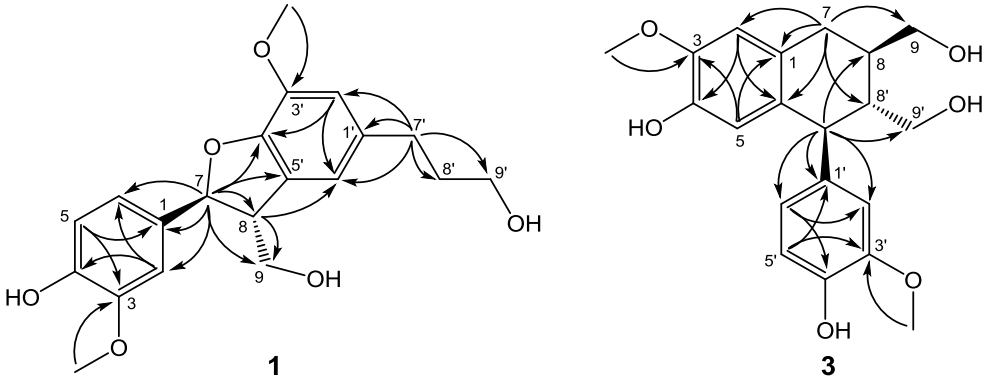

Figure 2: Key HMBC correlations of compounds **1** and **3**

Compound **4** was obtained as a white amorphous powder. The ESI-MS spectra of **4** exhibited an ion peak $[M + H]^+$ at *m/z* 421, which is in agreement with the molecular formula $C_{22}H_{28}O_8$. Its NMR data is very similar to those of **3**, except for the appearance of two methoxy group at C-5 and C-5′ position. The ^{1}H-NMR spectrum showed an aromatic proton signal at δ 6.61 (1H, s, H-6) attributed to benzene A ring, and a singlet signal of two other aromatic protons at δ 6.40 (2H, s, H-2′, 6′) were due to benzene B ring. The small coupling constant (*J* = 5.5 Hz) between H-7′ and H-8′ observed in the ^{1}H-NMR spectrum, established that the relative configuration of H-7′ and H-8′ was *cis*. Moreover, the absolute configuration of **4** was determined on the basis of the good agreement of NMR spectral data with those of polystachyol [10]. Thus, **4** was deduced as polystachyol.

Compound **5** was isolated as colorless oil. Its molecular formula was established as $C_{20}H_{22}O_6$ on the basis of an ion peak $[M+Na]^+$ at *m/z* 381 in ESI-MS. In the ^{13}C-NMR there were signals for only ten carbons, suggested that **5** might be a symmetrical structure. The ^{1}H-NMR spectrum showed signals for six aromatic protons of an ABX spin system at δ 6.97 (2H, d, 2.0, H-2, 2′), δ 6.79 (2H, d, 8.0, H-5, 5′), δ 6.83 (2H, dd, 8.0; 2.0, H-6, 6′), six protons of two methoxy group at δ 3.88 (6H, s, 3, 3′-OMe), two protons of two oximethine group at δ 4.73 (2H, d, 4.0, H-7, 7′), two protons of two methine group at δ 3.17 (2H, m, H-8, 8′), and four protons of two oxymethylene groups at δ 3.86 (2H, dd, 9.0, 3.5, H_a-9, 9′) and 4.25 (2H, dd, 9.0, 7.0, H_b-9, 9′). The above data indicated that compound **5** was assigned to be a lignan of the furofurane type. By comparing the NMR spectral data with those reported in literature, the structure of **5** was determined as (+)-pinoresinol [11].

Table 3: The NMR data of compound **5**

C	$^{\#}\delta_C$	δ_C[a,b]	δ_H[a,c] (*J* = Hz)	**C**	$^{\#}\delta_C$	δ_C[a,b]	δ_H[a,c] (*J* = Hz)
1, 1′	134.62	133.82	-	6, 6′	120.07	120.07	6.83 (2H, dd, 8.0; 2.0)
2, 2′	111.05	111.02	6.97 (2H, d, 2.0)	7, 7′	87.08	87.55	4.73 (2H, d, 4.0)
3, 3′	148.78	149.14	-	8, 8′	55.68	55.37	3.17 (2H, m)
4, 4′	147.31	147.33	-	9, 9′	72.66	72.62	3.86 (2H, dd, 9.0, 3.5) 4.25 (2H, dd, 9.0, 7.0)
5, 5′	116.00	116.10	6.79 (2H, d, 8.0)	3, 3′-OMe	56.70	56.44	3.88 (6H, s)

[a]recored in CD_3OD, [b]125 MHz, [c]500 MHz, $^{\#}\delta_C$ of (+)-pinoresinol in acetone-d_6 [11].

4. CONCLUSION

From the MeOH extract of the fruit of *Rhizophora stylosa*, using column chromatography, five lignan compounds (−)-(7*R*,8*S*)-dihydrodehydrodiconiferyl alcohol (**1**), (7*S*,8*R*)-3,3′,5-trimethoxy-4′,7-epoxy-8,5′-neolignan-4,9,9′-triol (**2**), (+)-isolariciresinol (**3**), polystachyol (**4**), (+)-pinoresinol (**5**) were isolated. Based on 1D-NMR and 2D-NMR as well as comparison with published data, their chemical structures were elucidated. This is the first report of these compounds from this species.

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