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C-Glycosidic flavonoids from *Cassia occidentalis*

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Abstract

Three new *C*-glycosidic flavonoids, cassiaoccidentalins A, B and C, were isolated from aerial parts of *Cassia occidentalis*, and their structures with a 3-keto sugar were established on the basis of spectroscopic and chemical evidence. They showed signals of two conformers in their ^{1}H- and ^{13}C-NMR spectra due to hindered rotation around their *C*-glycosidic linkages, and the conformations in solution were analyzed by NMR spectroscopic analyses. © 1999 Elsevier Science Ltd. All rights reserved.

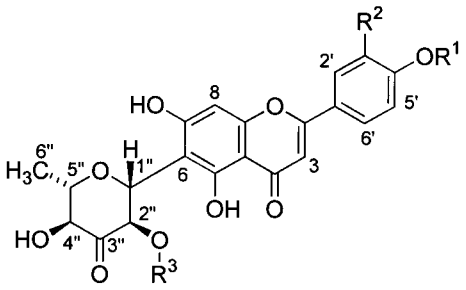
Keywords: Cassia occidentalis; Leguminosae; Cassiaoccidentalin A; Cassiaoccidentalin B; Cassiaoccidentalin C; Flavonoid; Conformation

1. Introduction

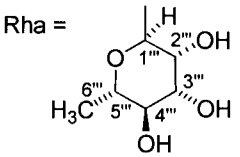
Certain plant species belonging to the genus *Cassia* (Leguminosae) have been used for medicinal purposes in Asian countries (Perry, 1980). Previously, we reported the isolation of flavan dimers and related phenolic constituents with lipase-inhibitory activity from *Cassia nomame* (Hatano et al., 1997). *Cassia occidentalis* L. has also been used as a mild laxative and a tonic in Japan and China (Mitsuhashi, 1988), and anthraquinones, xanthones and flavonoids such as matteucinol 7-*O*-rhamnoside have been reported as constituents (Glasby, 1991; Tiwari & Singh, 1977). The present investigation on the phenolic constituents of *C. occidentalis* revealed this plant species contains new *C*-glycosidic flavonoids with a 3-keto sugar. This paper deals with the structures and conformations of the new compounds.

2. Results and discussion

Chromatographic separation of the EtOAc soluble portion of the 70% acetone extract from the aerial parts of *C. occidentalis* gave three new compounds named cassiaoccidentalins A (**1**), B (**2**) and C (**3**), along with torosaflavone B 3′-*O*-glucoside which was previously isolated from *C. torosa* (Kitanaka & Takido, 1992).



1: $R^1 = R^2 = H$, R^3= Rha
2: R^1= H, R^2= OH, R^3= Rha
3: $R^1 = CH_3$, R^2=OH, R^3= Rha
4: $R^1 = R^2 = R^3$= H
5: $R^1 = CH_3$, R^2= OH, R^3= H



Structural formulae

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Table 1
^{13}C Chemical shifts of the major conformer of **1** and protons correlated with the carbon signals in the HMQC and HMBC spectra[a]

Carbon	δ_C	Proton coupled via one bond (δ_H)	Proton coupled via two or three bonds
Aglycone			
C-2	163.9		H-3, H-2′, H-6′
C-3	103.3	6.74	
C-4	182.4		H-3
C-4a	103.1		H-8
C-5	161.1		H-1″
C-6	107.8		H-8, H-1″
C-7	162.3		H-8, H-1″
C-8	93.5	6.53	
C-8a	156.9		H-8
C-1′	121.4		H-3, H-3′, H-5′
C-2′, C-6’	128.8	7.88	H-3′, H-5′
C-3′, C-5′	116.3	6.92	
C-4′	161.3		H-2′, H-6′, H-3′, H-5′
6-Deoxy-*ribo*-hexos-3-ulose			
C-1″	73.6	4.83	H-2″
C-2″	75.8	5.27	H-1″, H-1‴
C-3″	206.2		H-1″, H-4″
C-4″	78.2	3.88	
C-5″	78.4	3.37	H-1″, H-4″, H-6″
C-6″	19.2	1.28	H-4″
Rhamnose			
C-1‴	99.5	4.63	H-2″, H-2‴
C-2‴	70.4	3.69	
C-3‴	70.3	3.02	H-1‴, H-2‴, H-4‴
C-4‴	71.4	2.95	H-2‴, H-6‴
C-5‴	69.1	2.34	H-1‴, H-4‴, H-6‴
C-6‴	17.6	0.65	H-4‴

[a] 500 MHz for ^1H, and 126 MHz for ^{13}C, in DMSO-d_6 containing D_2O at 40°C.

Cassiaoccidentalin A (**1**) was obtained as pale-yellow needles, and ESIMS gave a $[M + H]^+$ ion peak at m/z 561. Its molecular formula was determined as $C_{27}H_{28}O_{13}$ by high-resolution (HR) ESIMS. The absorption maxima at 215, 271 and 336 nm in the UV spectrum are attributed to a flavone skeleton. Although the ^1H-NMR spectrum of **1** was complicated due to duplication or broadening of some of the signals even at elevated temperatures (40–50°C), the spectrum measured at 50°C indicated that this compound is composed of 6-*C*- (or 8-*C*-) substituted apigenin [δ 6.72 (1H, *br s*, H-3), 6.53 and 6.53 (1H in total, each *s*, A-ring H), 6.92 (2H, *d*, J = 9 Hz, H-3′ and H-5′), 7.88 (2H, *d*, J = 9 Hz, H-2′ and H-6′)] and two monosaccharide residues [δ 4.84 (1H, *d*, J = 10 Hz, H-1″) and 4.64 (1H, *br s*, H-1‴)]. The spectrum also showed signals corresponding to two methyl groups [δ 1.29 (3H, *d*, J = 6 Hz, H-6″′ 0.67 and 0.78 (3H in total, *br s*, H-6‴)], suggesting that the sugar residues are rhamnose or have a structure related to rhamnose.

An additional spectral complication, which is ascribable to a rotational barrier around the *C*-glycosidic linkage, was also observed in the ^{13}C-NMR spectrum of **1**. Moreover, the ^{13}C-NMR spectrum of **1** was similar to the data reported for apimaysin, although spectral complication for that compound was not mentioned (Snook et al., 1993). Chemical shifts of the aglycone carbons of **1** (shown in Table 1) are comparable with the corresponding carbons of 6-*C*-substituted apigenins such as apimaysin and torosaflavone A (Kitanaka & Ogata, 1989), indicating a C-6 substituted structure. The C-6 substitution in **1** was substantiated by a cross peak between H-8 and H-2′/H-6′ in the NOESY spectrum of.[1] The ^{13}C-signals at δ 99.5 (C-1‴), 70.4 (C-2‴), 70.3 (C-3‴), 71.4 (C-4‴), 69.1 (C-5‴), 17.6 (C-6‴) (for the major conformer) indicated that one of the monosaccharides in **1** is rhamnose with an *O*-glycosidic structure as in the case of apimaysin. The presence of rhamnose in **1** was substantiated by acid hydrolysis of **1** to give rhamnose along with **4**.

The ^{13}C-NMR spectrum of **1** also indicated that the remaining sugar residue has a ketone carbon (δ 206.2) along with one methyl (δ 19.2) and four methine (δ 73.6, 75.8, 78.2 and 78.4) carbons (for the major conformer). Apimaysin and its analogs have a 6-deoxypyranose with a ketone carbon (6-deoxy-*xylo*-hexos-4-

[1] This argument was based on a suggestion by one of the reviewers of this paper, who is thankfully acknowledged.

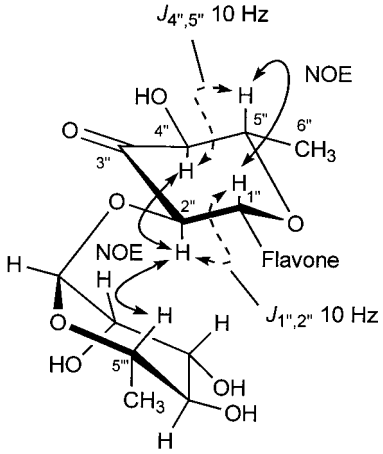

Fig. 1. Observed ^1H–^1H couplings and NOEs for protons of the 3-keto sugar residue in the molecule of cassiaoccidentalins A (**1**).

ulose) (Snook et al., 1993). However, the ^1H–^1H COSY spectrum of **1** showed two series of correlations, H-1″–H-2″ and H-4″–H-5″–H-6″ (CH_3). Therefore, the ketone carbon in **1** was at C-3″ of the pyranose, rather than at C-4″. The 3-keto structure for the sugar residue was further substantiated by HMBC correlations concerning the sugar including C-3″–H-1″ and C-3″–H-4″ as shown in Table 1. Additionally, the ^1H-NMR spectrum of **1** showed that the coupling constants $J_{1'',2''}$ and $J_{4'',5''}$ were both 10 Hz, indicating trans-diaxial relationships for H-1″–H-2″ and H-4″–H-5″. The NOESY spectrum of **1** showed correlations H-1″–H-5″ and H-2″–H-4″. Therefore, H-1″–H-5″ and H-2″–H-4″, are respectively on the same side of the pyranose ring as shown in Fig. 1. The structure of 6-deoxy-*ribo*-hexos-3-ulose was thus assigned for the sugar. The configuration of the sugar residue was assigned as shown in Fig. 1 based on the assumption that the rhamnose has the L-configuration, since the spectrum showed an NOE correlation between H-2″ and rhamnose H-5‴.

The HMBC spectrum also showed a correlation δ_H 4.63 (H-1‴)–δ_C 75.8 (C-2″), indicating that the rhamnose residue is attached to O-2″ of the 3-keto sugar residue. Based on these data, structure **1** was assigned for cassiaoccidentalin A. The NOESY spectrum of **1** showed a cross peak between H-8 of the aglycone and H-6‴ of the rhamnose residue for the major conformer, and a cross peak between aglycone OH-5 and rhamnose H-2‴ for the minor conformer, in addition to NOEs described above. Conformers exemplified in Fig. 2 satisfied the observed NOE correlations. Since the ^1H- and ^{13}C-NMR spectra of **4** did not show spectral complication due to duplication and broadening of signals, the complication observed in the spectra of **1** is largely dependent on the presence of the rhamnose residue at O-2″.

Cassiaoccidentalin B (**2**) was obtained as pale-yellow

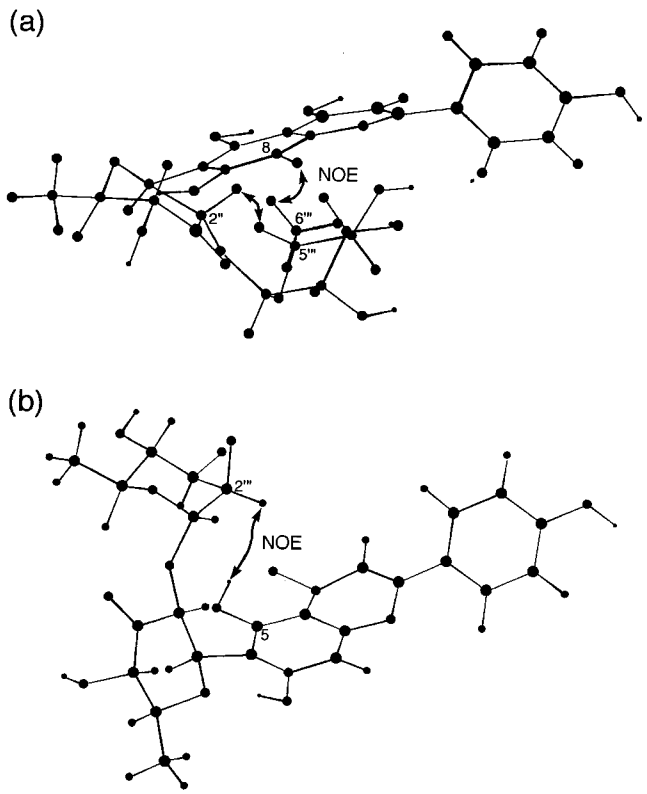

Fig. 2. Plausible conformations for the major (a) and minor (b) conformers of cassiaoccidentalin A (**1**).

needles. The ESIMS showed an $[M + H]^+$ ion peak at m/z 577, which was 16 mass units larger than that for **1**, suggesting the molecular formula $C_{27}H_{28}O_{14}$ for **2**. The UV spectrum of **2** was similar to that of luteolin rather than apigenin (Mabry, Markham & Thomas, 1970). The ^1H- and ^{13}C-NMR spectra were also complicated by the presence of conformational isomers as for **1**. Although sugar proton signals in the ^1H-NMR spectrum of **2** were similar to those of **1**, the aglycone protons at δ 6.50 (1H, *s*, H-8), 6.64 (1H, *br s*, H-3), 6.88 (1H, *d*, J = 8.5 Hz, H-5′), 7.38 (2H, *m*, H-2′ and H-6′) indicated that **2** has a luteolin residue as the aglycone. The C-6 substitution of the aglycone was based on comparison of ^{13}C chemical shifts of the aglycone moiety with the corresponding signals of 6-*C*-substituted flavones including maysin (Snook et al., 1993). Structure **2**, in which a luteolin residue is substituted for the apigenin residue of **1**, was thus assigned for cassiaoccidentalin B.

Cassiaoccidentalin C (**3**) was also obtained as pale-yellow needles. The ESIMS showed an $[M + H]^+$ ion peak at m/z 591, corresponding to the molecular formula $C_{28}H_{30}O_{14}$, 14 mass units larger than that of **2**. The ^1H-NMR spectrum of **3** showed a methoxyl signal at δ 3.84 (3H, *s*), in addition to the proton signals of the aglycone at δ 6.72 (H-3), 6.53 (H-8), 7.40 (H-2′),

7.07 (H-5′) and 7.51 (H-6′), indicating that the B-ring of the aglycone has a methoxyl group at C-3′ or C-4′. An analogous pattern of sugar proton resonances indicated that **3** has the same sugar residue as **1**. Treatment of **3** with dil. HCl gave **5** along with rhamnose. The NOESY spectrum of **5** showed a correlation between the methoxyl group and H-5′, thus establishing the location of the methoxyl group at C-4′′. Structure **3** was, therefore, assigned to cassiaoccidentalin C. The ^{13}C-NMR spectral data of **3** shown in Section 3 were consistent with this structure.

As far as we know, this is the first report of flavonoids with a 6-deoxy-*ribo*-hexos-3-ulosyl residue.

3. Experimental

3.1. General

^{1}H- and ^{13}C-NMR spectra were recorded on a Varian VXR500 instrument (500 MHz for ^{1}H and 126 MHz for ^{13}C) at 40°C, and DMSO-d_6 containing D_2O (ca. 5%) was used for solvent unless otherwise mentioned. Chemical shifts were based on those of the solvent signals (δ_H 2.48 and δ_C 39.7 for DMSO-d_6, and δ_H 3.30 for CD_3OD) and given in δ (ppm) values from TMS. The HMBC spectrum was measured with the GHMBC pulse sequence. ESIMS were recorded on a Micromass Autospec OA-Tof spectrometer, and the solvent used for loading samples was 50% MeOH containing 0.1% $AcONH_4$. TLC was performed on silica gel with $CHCl_3$–MeOH–H_2O (16 : 9 : 2 by volume), and spots were visualized by spraying with a mixture of thymol in EtOH (0.5 g/95 ml) and conc. H_2SO_4 (5 ml) (Ceska & Styles, 1984).

3.2. Isolation of flavonoids from C. occidentalis

Dried above ground parts of *C. occidentalis* (250 g), cultivated in Tokushima prefecture, Japan (purchased from Tochimoto-tenkai-do, Japan), were homogenized in 70% acetone (2 l × 3), and the insoluble material was removed by filtration. The solution was concentrated in vacuo (to 500 ml), and then extracted with Et_2O (500 ml × 5) and EtOAc (500 ml × 4), successively. The EtOAc extract (2 g) was chromatographed over Toyopearl HW-40F (Toso) with 70% EtOH, and a fraction containing flavonoids was further purified by CC on MCI gel CHP-20P (Mitsubishi Chemical Industries) with aqueous MeOH, and on SepPak C_{18} (Waters) or MCI gel CHP-20P, to give cassiaoccidentalins A (**1**) (31 mg), B (**2**) (43 mg) and C (**3**) (22 mg). The EtOAc extract (7.5 g) obtained from 1 kg of the aerial parts was treated in an analogous way, to give torosaflavone B 3′-glucoside (11 mg), together with cassiaoccidentalins.

3.3. Cassiaoccidentalin A (*1*)

Pale-yellow needles, mp 175°C (from MeOH–H_2O). $[\alpha]_D$ −80.1° (MeOH; *c* 1). ESIMS *m/z*: 561 ([M + H]$^+$). HR-ESIMS *m/z*: 561.1616 ([M + H]$^+$). Calcd. for $C_{27}H_{28}O_{13}$ + H *m/z*: 561.1608. UV λ_{max} (MeOH) nm (log ϵ): 215 (4.57), 271 (4.38), 336 (4.42). ^{1}H-NMR spectral data (50°C): δ 0.67 [major conformer (Mj)], 0.78 [minor conformer (mi)] (3H in total, *br s*, H-6‴ × 3), 1.29 (3H, *d*, *J* = 5.5 Hz, H-6″ × 3), 2.34 (Mj), 2.41 (mi) (1H in total, *m*, H-5‴), 2.95 (1H, *t*, *J* = 9.5 Hz, H-4‴), 3.02 (1H, *m*, H-3‴), 3.37 (1H, *m*, H-5″), 3.69 (1H, *br m*, H-2‴), 3.88 (1H, *d*, *J* = 10 Hz, H-4″), 4.63 (1H, *d*, *J* = 1 Hz, H-1‴), 4.83 (1H, *d*, *J* = 10 Hz, H-1″), 5.22 (mi), 5.27 (Mj) (1H in total, *br d*, *J* = 10 Hz, H-2″). Aglycone protons, see text. ^{13}C-NMR spectral data (40°C), see Table 1 for the major conformer. Signals due to the minor conformer: δ 17.9 (C-6‴), 76.3 (C-2″), 73.2 (C-1″), 94.3 (C-8), 104.0 (C-4a), 107.6 (C-6), 157.1 (C-8a), 160.0 (C- 5), 163.4 (C-7), 182.1 (C-4). The other signals are overlapped with the signals of the major conformer.

3.4. Cassiaoccidentalin B (*2*)

Pale-yellow needles, mp 194°C (from MeOH–H_2O). $[\alpha]_D$ −63.6° (MeOH; *c* 1). ESIMS *m/z*: 577 ([M + H]$^+$). HR-ESIMS *m/z*: 577.1575 ([M + H]$^+$). Calcd. for $C_{27}H_{28}O_{14}$ + H *m/z*: 577.1557. UV λ_{max} (MeOH) nm (log ϵ): 211 (4.43), 229 (sh), 245 (4.13), 258 (4.16), 270 (4.16), 350 (4.22). ^{1}H-NMR spectral data, see text. ^{13}C-NMR spectral data for the major conformer: δ 17.6 (C-6‴), 19.3 (C-6″), 69.2 (C-5‴), 70.4 (C-3‴), 70.5 (C-2‴), 71.5 (C-4‴), 73.6 (C-1″), 75.9 (C-2″), 78.3 (C-4″), 78.5 (C-5″), 93.5 (C-8), 99.5 (C-1‴), 103.3 (C-3), 103.9 (C-4a), 107.8 (C-6), 113.5 (C-2′), 116.4 (C-5′), 119.4 (C-6′), 121.7 (C-1′), 145.9 (C-3′), 149.9 (C-4′), 156.9 (C-8a), 161.2 (C-5), 163.6 (C-7), 164.0 (C-2), 182.4 (C-4), 206.3 (C-3″).

3.5. Cassiaoccidentalin C (*3*)

Pale-yellow needles, mp 193°C (from MeOH–H_2O). $[\alpha]_D$ −55.6° (MeOH; *c* 1). ESIMS *m/z*: 591 ([M + H]$^+$). HR-ESIMS *m/z*: 591.1876 ([M + H]$^+$). Calcd. for $C_{28}H_{30}O_{14}$ + H *m/z*: 591.1714. UV λ_{max} (MeOH) nm (log ϵ): 211 (4.41), 243 (4.13), 253 (4.12), 271 (4.16), 340 (4.21). ^{1}H-NMR spectral data, see text. ^{13}C-NMR spectral data for the major conformer: δ 17.5 (C-6‴), 19.2 (C-6″), 69.2 (C-5‴), 70.3 (C-3‴), 70.4 (C-2‴), 71.5 (C-4‴), 73.6 (C-1″), 75.8 (C-2″), 78.2 (C-4″), 78.4 (C-5″), 93.4 (C-8), 99.4 (C-1‴), 103.4 (C-3), 104.0 (C-4a), 107.8 (C-6), 112.6 (C-2′), 113.1 (C-5′), 119.1 (C-6′), 123.1 (C-1′), 147.0 (C-3′), 151.5 (C-4′), 156.9 (C-8a), 161.1 (C-5), 163.6 (C-7), 163.6 (C-2), 182.3 (C-4), 206.1 (C-3″).

3.6. Hydrolysis of cassiaoccidentalins A (*1*) and C (*3*)

Aqueous HCl (1%, 1 ml) was added to cassiaoccidentalin A (**1**) (10 mg), and the mixture in a sealed tube was heated on a boiling-water bath for 1 h. Aglycone (**4**) (4 mg) was obtained from the precipitate formed upon the reaction and also from the supernatant after chromatography on a Mega-BondElute cartridge (Varian) with H_2O and then with aqueuos MeOH. The material, which passed through the Mega-BondElute cartridge with water, was concentrated and subjected to TLC, indicating the presence of rhamnose (as a reddish yellow spot at R_f 0.28). Cassiaoccidentalin C (**3**) (21 mg) was treated in an analogous way to give **5** (5 mg). Production of rhamnose upon the hydrolysis of **3** was also demonstrated by TLC.

3.6.1. Compound *4*

UV λ_{max} (MeOH) nm (log ϵ): 215 (4.33), 271 (4.30), 331 (4.38). ESIMS m/z: 415 ($[M + H]^+$). HR-ESIMS m/z: 415.1001 ($[M + H]^+$). Calcd. for $C_{21}H_{18}O_9$ + H, 415.1029. ^{1}H-NMR spectral data: δ 1.28 (3H, *d*, J = 6 Hz, H-6″), 3.34 (1H, *m*, H-5″, in part overlapped with a HDO signal), 3.86 (1H, *d*, J = 10 Hz, H-4″), 4.68 (1H, *d*, J = 10 Hz, H-1″), 5.07 (1H, *d*, J = 10 Hz, H-2″), 6.54 (1H, *s*, H-8), 6.74 (1H, *s*, H-3), 6.92 (2H, *d*, J = 9 Hz, H-3′ and H-5′), 7.89 (2H, *d*, J = 9 Hz, H-2′ and H-6′). ^{13}C-NMR spectral data: δ 19.3 (C-6″), 73.2 (C-2″), 75.3 (C-1″), 78.0 (C-4″), 78.6 (C-5″), 93.8 (C-8), 103.0 (C-4a), 103.2 (C-3), 107.9 (C-6), 116.2 (C-3′ and C-5′), 121.4 (C-1′), 128.7 (C-2′ and C-6′), 156.8 (C-8a), 160.0 (C-5), 161.2 (C-4′), 162.2 (C-7), 164.0 (C-2), 182.2 (C-4), 207.7 (C-3″).

3.6.2. Compound *5*

UV λ_{max} (MeOH) nm (log ϵ): 214 (4.33), 254 (4.10), 270 (4.11), 346 (4.02). ESIMS m/z: 445 ($[M + H]^+$). HR-ESIMS m/z: 445.1158 ($[M + H]^+$). Calcd. for $C_{22}H_{20}O_{10}$ + H, 445.1135. ^{1}H-NMR spectral data (in CD_3OD, 40°C): δ1.44 (3H, *d*, J = 6.5 Hz, H-6″), 3.48 (1H, *m*, H-5″), 4.03 (1H, *dd*, J = 2, 10 Hz, H-4″), 4.92 (1H, *d*, J = 10 Hz, H-1″), 5.28 (1H, *dd*, J = 2, 10 Hz, H-2″), 6.51 (1H, *s*, H-8), 6.59 (1H, *s*, H-3), 7.06 (1H, *d*, J = 8.5 Hz, H-5′), 7.38 (1H, *d*, J = 2 Hz, H-2′), 7.47 (1H, *dd*, J = 2, 8.5 Hz, H-6′).

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