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Japonenynes, halogenated C_{15} acetogenins from *Laurencia japonensis*

Yoshinori Takahashi[a], Minoru Suzuki[a,*], Tsuyoshi Abe[b], Michio Masuda[b]

[a] *Division of Material Science, Graduate School of Environmental Earth Science, Hokkaido University, Sapporo 060-0810, Japan*
[b] *Division of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo 060-0810, Japan*

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Abstract

Two brominated C_{15} nonterpenoid compounds, japonenyne-A and -B, with a novel 2,7-dioxabicyclo[4.3.0]nonane skeleton have been isolated from the red alga *Laurencia japonensis* Abe et Masuda. The structures of these metabolites were deduced from spectral evidence.

Keywords: *Laurencia japonensis*; Rhodomelaceae; Red alga; Acetogenin; Halogenated compound; Chemotaxonomy

1. Introduction

In continuing our chemotaxonomical studies on Japanese species of the red algal genus *Laurencia*, we have recently reported that *L. japonensis* (Abe & Masuda, 1998), collected from several locations in Japanese waters, contains aplysiadiol (**1**) (diterpenoid) as a major metabolite along with 2,10-dibromo-3-chloro-α-chamigrene (**2**) (sesquiterpenoid), a set of halogenated compounds characteristic to this species (Takahashi, Suzuki, Abe, & Masuda, 1998).

Further investigation of the methanol extracts of this species led to the isolation of two novel halogenated C_{15} nonterpenoids, designated as japonenyne-A (**3**) and -B (**4**), along with japonenyne-C (**5**). Japonenyne-A (**3**) and -B (**4**) consist of 2,7-dioxabicyclo[4.3.0]nonane skeleton with the same molecular formula of $C_{15}H_{18}Br_2O_2$ as that of laurenenyne-A (**6**) and -B (**7**), which have previously been obtained from this species collected at Kamishima, Mie Prefecture, Japan (Suzuki, Matsuo, & Masuda, 1993; Takahashi et al., 1998). We wish to report herein the isolation and structural elucidation of these novel halogenated metabolites.

2. Results and discussion

Chromatographic separation of the methanol extracts obtained from *L. japonensis* collected at several locations in Japanese waters has led to the isolation of three halogenated C_{15} nonterpenoids, **3**–**5**, together with aplysiadiol (**1**), which has previously been isolated from a Japanese sea hare *Aplysia kurodai* (Ojika, Yoshida, Okumura, Ikeda, & Yamada, 1990), and 2,10-dibromo-3-chloro-α-chamigrene (**2**), which has already been found in other *Laurencia* species (Howard & Fenical, 1975; Suzuki, Furusaki, & Kurosawa, 1979; Suzuki, Segawa, Kikuchi, Suzuki, & Kurosawa, 1985). These C_{15} nonterpenoids, which exist as colorless compounds when developed on Si gel TLC plate, turned to characteristic dark red-colored spots when sprayed with 5% phosphomolybdic acid in ethanol and subjected to heating. These compounds were also observed to be very unstable and decomposed even when stored at $-18^{\circ}C$ for just a few days.

Japonenyne-A (**3**), colorless oil, $[\alpha]^{21}_{D}$ $+40.2^{\circ}$ (*c* 1.39; CH_2Cl_2), was shown to have the molecular formula of $C_{15}H_{18}Br_2O_2$ by a high-resolution mass spectrum (*m*/*z* 389.9673 [M], $\Delta+2.0$ mmu). The IR spectrum showed the presence of terminal acetylenic (ν_{max} 3310 cm^{-1}) and vinyl ethereal (1690 and 1205 cm^{-1}) functionalities. The presence of a 2-penten-4-ynyl moiety, which is frequently encountered in *Laurencia*'s C_{15} acetogenin, was readily recognized by the ^1H NMR spectrum (Table 1) [δ_H 2.85 (1H, d, $J=1.5$ Hz), 5.57 (1H, dddd, $J=16.1$, 1.5, 1.5 and 1.5 Hz) and 6.19 (1H, ddd, $J=16.1$, 6.4 and 6.4 Hz)]. The magnitude of coupling constants ($J_{3,4}=16.1$ Hz) for the H-3 and H-4 as well as the chemical shift value (δ_H 2.85) of the acetylenic proton indicated the geometry of the double bond at C-3 to be *E* (Suzuki & Kurosawa, 1987).

* Corresponding author. Tel.: +81-11-706-2272; fax: +81-11-709-6416; e-mail: misu@ees.hokudai.ac.jp.

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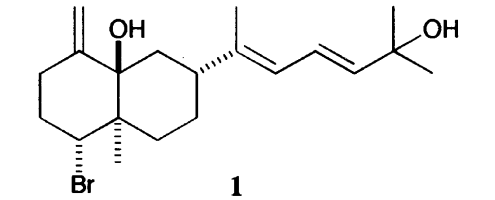

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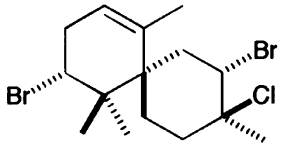

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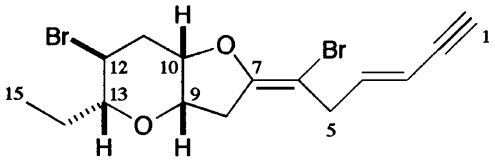

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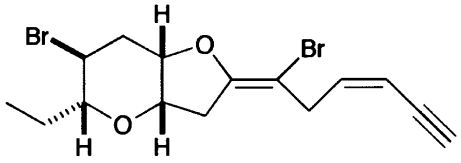

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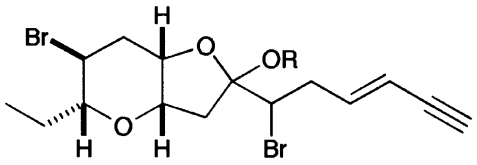
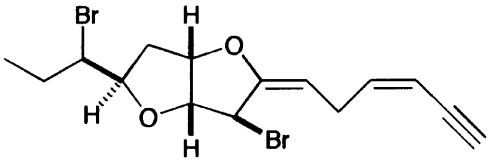


5: R=CH_3
8: R=H



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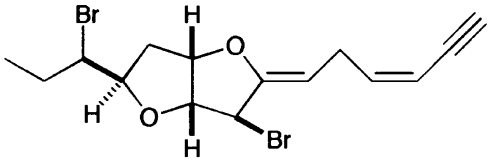

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Table 1
^{13}C (100 MHz, DEPT) and ^{1}H NMR (400 MHz) and HMBC data[a] for japonenyne-A (**3**)

C[b]	^{13}C (δ)	^{1}H (δ)	*J* (Hz)	Long-range correlations
1	[c]	2.85	d (1.5)	
2	83.49			H-4
3	110.19	5.57	dddd (16.1, 1.5, 1.5, 1.5)	H-1, H_2-5
4	141.64	6.19	ddd (16.1, 6.4, 6.4)	H_2-5
5	38.90	3.13	m	H-4
6	88.44			H-4, H_2-5, H_β-8
7	152.58			H_2-5, H_α-8, H-9
8	36.76	2.69	d (16.6 (H_α))	
		2.62	dd (16.6, 3.9 (H_β))	
9	75.68	4.26	dd (3.9, 2.4)	H_α-8, H_α-11
10	89.91	4.21	ddd (3.9, 2.4, 2.4)	H_α-8, H-9, H_α-11
11	37.33	2.94	ddd (14.6, 4.4, 2.4 (H_α))	H-13
		2.22	ddd (14.6, 12.2, 3.9 (H_β))	
12	46.38	3.97	ddd (12.2, 9.3, 4.4)	H_2-11
13	80.96	3.31	ddd (9.3, 8.3, 2.4)	H-12, H_3-15
14	26.13	2.01	ddq (14.6, 2.4, 7.3 (H_a))	H_3-15
		1.54	ddq (14.6, 8.3, 7.3 (H_b))	
15	9.22	0.94	t (7.3)	H_a-14

[a] Measured in chloroform-d_1.
[b] All assignments are based on the results of the HSQC spectrum.
[c] This carbon was not observed in the ^{13}C NMR spectrum.

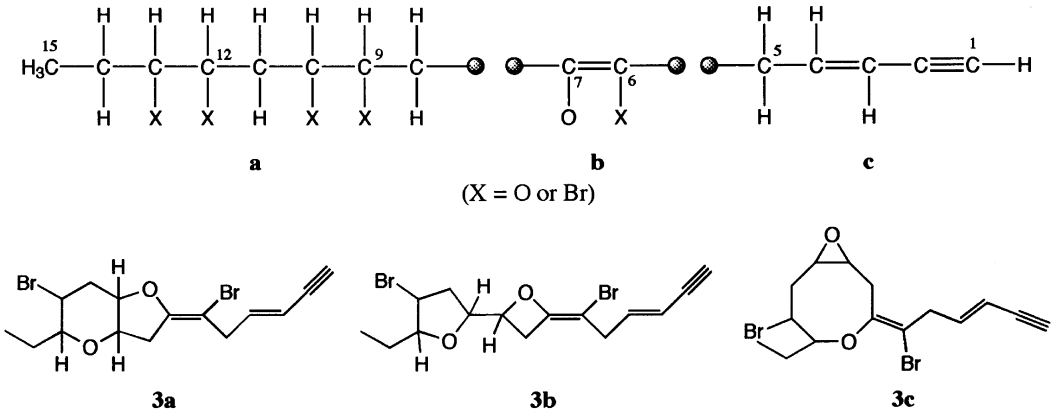


Fig. 1. Partial structural units and possible structures for **3**.

Moreover, japonenyne-B (**4**), colorless oil, $[\alpha]^{21}_{D}$ +24.4° (*c* 0.61; CH_2Cl_2), was an isomeric metabolite of japonenyne-A (**3**). The ^1H and ^{13}C NMR signals (Table 2) on the carbons from C-8 to C-15 in both compounds **4** and **3** were virtually identical. Comparison of the spectral data of **4** and **3** suggested that japonenyne-B is a geometric isomer with *Z*-configuration at C-3.

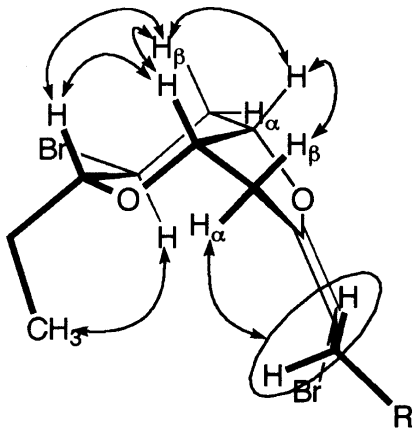
Detailed analysis of the ^1H and ^{13}C NMR, ^1H-^1H COSY and HSQC spectra of **3** revealed the presence of partial structural units **a** and **c** (Figure 1). In the ^{13}C NMR spectrum, the chemical shift (δ_C 46.38) of the methine carbon at C-12 in the unit **a** indicated that a bromine atom is attached to this carbon. Furthermore, since the IR spectrum showed no absorption ascribable to an hydroxyl group, the remaining substituents at C-9, C-10 and C-13 were verified to be ethereal oxygen atoms based upon the chemical shift values of the pertinent carbons at δ_C 75.68, 89.91 and 80.96, respectively. As described above, in the IR spectrum the intense absorption at ν_{max} 1690 and 1205 cm^{-1} suggested the presence of a trisubstituted vinyl ether grouping (partial unit **b**) which was further supported by the signals of two quaternary carbons at δ_C 152.58 and 88.44 in the ^{13}C NMR spectrum. Since the C_{15} nonterpenoid cyclic ethers of *Laurencia* species seem to be biosynthesized from straight-chain precursors, viz. laurediols from *L. nipponica* (Kikuchi, Suzuki, Kurosawa, & Suzuki, 1991), the unit **b** necessitates being linked to C-8 at one end and to C-5 at the other end. Judging from the chemical shift value of δ_C 88.44, the fourth substituent of the unit **b** must obviously

Table 2
^{13}C (100 MHz, DEPT) and ^1H NMR (400 MHz) and HMBC data[a] for japonenyne-B (**4**)

C[b]	^{13}C (δ)	^1H (δ)	*J* (Hz)	Long-range correlations
1	80.20	3.12	d (2.0)	
2	82.14			H-5
3	109.35	5.56	br dd (10.7, 2.0)	H_2-5
4	141.80	6.07	ddd (10.7, 7.3, 6.8)	H_2-5
5	36.44	3.43	dd (16.1, 7.3)	
		3.30	dd (16.1, 6.8)	
6	89.73			H_2-5, H_2-8
7	152.07			H_2-5, H_2-8
8	37.03	2.81	d (16.6 (H_α))	
		2.68	dd (16.6, 4.4 (H_β))	
9	75.84	4.25	dd (4.4, 2.0)	H_α-8
10	80.92	4.19	ddd (3.9, 2.4, 2.0)	H_α-8, H-9
11	37.46	2.93	ddd (14.7, 4.4, 2.4 (H_a))	
		2.21	ddd (14.7, 11.7, 3.9 (H_β))	
12	46.53	3.97	ddd (11.7, 10.3, 4.4)	H_β-11
13	81.04	3.30	ddd (10.3, 8.3, 2.4)	H_α-11, H_b-14, H_3-15
14	26.22	2.01	ddq (14.6, 2.4, 7.3 (H_a))	H_3-15
		1.52	ddq (14.6, 8.3, 7.3 (H_b))	
15	9.25	0.94	t (7.3)	H_2-14

[a] Measured in chloroform-d_1.
[b] All assignments are based on the results of the HSQC spectrum.

not be oxygen but bromine. Then in order to prove the planar structure, we measured ^{1}H-detected heteronuclear multiple-bond ^{1}H–^{13}C correlation spectrum (HMBC). In the HMBC spectrum Table 1, the H-4 (δ_H 6.19) showed a cross peak to the quaternary carbon at δ_C 88.44 and the H-9 (δ_H 4.26) showed a cross peak to the quaternary carbon at δ_C 152.58, thus revealing that the carbon of δ_C 88.44 (vinyl bromide carbon) is linked to C-5 and hence that of δ_C 152.58 to C-7. Furthermore, since japonenyne-A (**3**) has six degrees of unsaturation, **3** has to contain two oxide rings, leading to three possible planar structures **3a**, **3b** and **3c** Fig. 1 assignable for japonenyne-A. The structure **3c**, however, could easily be ruled out because the ^{1}H NMR spectrum exhibited no signals due to a 1,2-disubstituted oxirane ring (Tori, Komeno, & Nakagawa, 1964). Furthermore, in the ^{1}H NMR spectrum the protons on the carbons from C-9 to C-13 showed coupling constants of $J_{9,10}=2.4$ Hz, $J_{10,11\alpha}=2.4$ Hz, $J_{10,11\beta}=3.9$Hz, $J_{11\alpha,12}=4.4$ Hz, $J_{11\beta,12}=12.2$ Hz and $J_{12,13}=9.8$ Hz, which are typical equatorial–equatorial, equatorial–axial and axial–axial coupling constants due to the protons not on a tetrahydrofuran ring but on a tetrahydropyran ring with a chair-like conformation. Thus japonenyne-A should have the planar formula **3a**. The geometry of the double bond between C-6 and C-7 in **3** was defined by the NOESY correlation spectrum. As shown in Fig. 2, the correlation between H_2-5 and H_α-8 indicated that the configuration of C-6 and C-7 is *Z*. Furthermore, the relative stereochemistry was also defined by the NOESY correlation spectrum. The NOEs between H_β-8/H-10, H-9/H_β-11, H-9/H-13, H-10/H_β-11, H_β-11/H-13, H_α-11/H-12 and H-12/H_3-15 showed that the relative configuration between H-9, H-10 and H-13 are all *cis*. Likewise the gross structure of japonenyne-B was confirmed with the aid of the 2-D NMR spectra, such as ^{1}H-^{1}H COSY, HSQC and HMBC Table 2. Moreover, the relative stereochemistry was also determined by the NOESY spectrum Fig. 2 which was very similar to that of **3**. Therefore, the structures of japonenyne-A and -B should be shown as formulas **3** and **4**, respectively, each of which includes the relative configuration of 9*S**, 10*S**, 12*S** and 13*R**.



3: R = *trans*-2-penten-4-ynyl moiety
4: R = *cis*-2-penten-4-ynyl moiety

Fig. 2. NOEs from NOESY spectra of japonenyne-A (**3**) and -B (**4**).

Many halogenated C_{15} acetogenins isolated from various *Laurencia* species are suggested to arise from (6*S*,7*S*)- or (6*R*,7*R*)-laurediol (Kikuchi et al., 1991). Japonenyne-A (**3**) may be biosynthesized from a bromohydrin **8** through an enzymatic E2-type dehydration. A bromohydrin **8** would be derived from (6*S*,7*S*)- or (6*R*,7*R*)-(3*E*)-laurediol via a bromonium ion-catalyzed cyclization and have a *threo* configuration at C-6 and C-7. The presence of a plausible precursor **8** should strongly be supported by the isolation of japonenyne-C (**5**), whose structure was determined by spectral analyses using extensive 2-D NMR such as ^{1}H-^{1}H COSY, HSQC and HMBC as in the case of **3** and **4**. The stereochemistry at C-6 and C-7, remains unsettled because compound **5** decomposed while measuring the NOESY spectrum. However, compound **5** was obtained, not as a mixture of diastereomers, but as a single compound, based on the single ^{1}H and ^{13}C NMR signals due to the bromomethine at C-6 and methoxyl methyl group, it may nevertheless be an artefact formed during extraction. Japonenynes represent the first example of halogenated C_{15} acetogenins found from various species of the red algal genus *Laurencia* possessing a 2,7-dioxabicyclo[4.3.0]nonane skeleton and a bromine atom substituent at C-6.

3. Experimental

3.1. General

The ^{1}H NMR: 400 MHz and ^{13}C NMR: 100 MHz, $CDCl_3$ or C_6D_6, TMS as int. standard; LR-MS and HR-MS: 70 eV; CC: silica gel (Merck, Kieselgel 60, 70–230 mesh); prep. TLC: silica gel plate (Merck, Kieselgel 60 F_{254S}).

3.2. Isolation

As recently described (Takahashi et al., 1998), the methanol extract (691 mg) of the Chinzei sample (SAP 062630) was fractionated by column chromatography on Si gel with a step gradient (hexane and EtOAc). The fraction (288 mg) eluted with hexane–EtOAc (9:1) was further subjected to prep. TLC with toluene to give japonenyne-A (**3**) (2.1 mg, 0.3%) and japonenyne-C (**5**) (16.6 mg, 2.4%) along with anhydroaplysiadiol (Takahashi et al., 1998).

Japonenyne-B (**4**) was obtained in 0.2% yield of the extract from Toyooka sample (SAP 062614) by combination of column and thin-layer chromatography in the same manner as described above. Japonenyne-A (**3**) has also been isolated from Toyooka and Heki sample

(SAP 062611) and japonenyne-B (**4**) from Iwami sample (SAP 062613) as the minor metabolite.

3.3. Japonenyne-A (**3**)

Colorless oil; $[\alpha]^{21}_{D}$ +40.2° (*c* 1.39; CH_2Cl_2); IR ν_{max} (CH_2Cl_2) cm^{-1}: 3310, 2940, 1690, 1420, 1205, 1180, 1130, 1100, 1040 and 960; ^1H and ^{13}C NMR Table 1; EI–LR-MS *m/z* (rel. int.): 392 (5.5), 390 (10.7), 388 (5.6) $[M]^+$, 311 (15.3), 309 (14.8) $[M–Br]^+$, 281 (10.8), 279 (10.2), 267 (17.8), 229 (17.5) $[M–2Br]^+$, 191 (22.9), 189 (23.5), 147 (57.3), 127 (37.7), 109 (73.7), 105 (100), 81 (56.4), 77 (65.8) and 41 (25.0); EI–HR-MS *m/z*: 389.9673. Calcd for $C_{15}H_{18}{}^{79}Br^{81}BrO_2$, 389.9652 [M].

3.4. Japonenyne-B (**4**)

Colorless oil, $[\alpha]^{21}_{D}$ +24.4° (*c* 0.61; CH_2Cl_2); IR ν_{max} (CH_2Cl_2) cm^{-1}: 3310, 2980, 1690, 1420, 1205, 1180, 1130, 1100, 1040, 950 and 920; ^1H NMR Table 2; EI–LR-MS *m/z* (rel. int.): 392 (0.3), 390 (0.7), 388 (0.4) $[M]^+$, 311 (1.7), 309 (1.8) $[M–Br]^+$, 310 (2.4), 308 (2.1) $[M–HBr]^+$, 281 (10.8), 279 (10.6), 253 (1.4), 251 (1.5), 239 (3.5), 273 (3.3), 216 (39.2), 201 (100), 159 (42.5), 145 (46.7), 115 (31.0), 82 (70.0) and 80 (68.0); EI–HR-MS *m/z*: 389.9672. Calcd for $C_{15}H_{18}{}^{79}Br^{81}BrO_2$, 389.9652 [M].

3.5. Japonenyne-C (**5**)

Colorless oil; $[\alpha]^{26}_{D}$ −35.5° (*c* 0.06; $CHCl_3$); IR ν_{max} ($CHCl_3$) cm^{-1}: 3330, 2940, 1545, 1460, 1435, 1210, 1110, 1040, 815 and 730; ^1H NMR (C_6D_6): δ 0.91 (3H, t, J=7.3 Hz; H_3-15), 1.56 (1H, ddq, J=14.2, 6.8, 7.3 Hz; H_b-14), 1.74 (1H, ddd, J=14.7, 11.7, 3.9 Hz; H_β-11), 1.84 (1H, dd, J=14.7, 5.4 Hz; H_β-8), 1.91 (1H, ddq, J=14.2, 2.9, 7.3 Hz; H_a-14), 2.37 (1H, d, J=14.7 Hz; H_α-8), 2.48 (1H, m; H_b-5), 2.52 (1H, ddd, J=14.7, 4.4, 2.4 Hz; H_α-11), 2.56 (1H, d, J=1.5 Hz; H-1), 2.80 (1H, m; H_a-5), 2.81 (3H, s; OMe), 3.01 (1H, ddd, J=9.8, 6.8, 2.9 Hz; H-13), 3.37 (1H, ddd, J=3.9, 2.4, 2.0 Hz; H-10), 3.42 (1H, dd, J=5.4, 2.0 Hz; H-9), 3.96 (1H, ddd, J=11.7, 9.8, 4.4 Hz; H-12), 4.17 (1H, dd, J=10.3, 3.4 Hz; H-6), 5.45 (1H, dddd, J=16.1, 1.9, 1.5, 1.5 Hz; H-3) and 6.32 (1H, ddd, J=16.1, 7.3, 7.3 Hz; H-4); ^{13}C NMR (C_6D_6): CH_3; δ 8.80 (C-15), 48.50 (OMe), CH_2; δ 26.50 (C-14), 36.58 (C-5), 37.73 (C-11), 40.64 (C-8), CH; δ 47.19 (C-12), 56.02 (C-6), 76.76 (C-9), 77.96 (C-1), 79.24 (C-10), 80.62 (C-13), 111.72 (C-3), 143.20 (C-4) and C; δ 109.58 (C-7); EI-LR-MS *m/z* (rel. int.): 424 (0.4), 422 (0.8), 420 (0.4) $[M]^+$, 393 (1.4), 391 (2.5), 389 (1.3) $[M–OMe]^+$, 343 (3.3), 341 (3.5) $[M–Br]^+$, 311 (25.5), 309 (25.0), 265 (33.0), 263 (33.2), 229 (10.7), 191 (16.6), 189 (17.8), 183 (36.5), 149 (35.0), 109 (100), 105 (86.9), 81 (66.3), 69 (54.3), 57 (76.6) and 41 (87.5); EI–HR-MS *m/z*: 423.9890. Calcd for $C_{16}H_{22}{}^{79}Br^{81}BrO_3$, 423.9894 [M].

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