



## Two New Flavanones from the Leaves of *Flemingia lineata* (L.) Aiton

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**Abstract** – Three isoprenylated flavanones were isolated from the leaves of *Flemingia lineata* (L.) Aiton. Among them are two new flavanones, flemilineatins A and B (**1** - **2**), along with 6-isoprenyl eridiocytol (**3**). Their structures were determined using HRESIMS data and NMR spectra. Flavanones **1** - **3** were assayed in the HeLa cancer cells. Compound **1** showed moderate activity with an IC<sub>50</sub> value of 11.2 μM.

**Keywords** – *Flemingia lineata*, flemilineatins A-B, flavanone, cytotoxicity

### Introduction

*Flemingia lineata* (L.) Aiton, belonging to the Fabaceae family, is a fast-growing shrub native to Southeast Asia and Australia. *F. lineata*, commonly called as otok-otok in Java, is used to soil erosion control and provide organic manure. The leaves also cure scabies, fever, and inflammation.<sup>1</sup> The *Flemingia* genus produces flavonoids and isoflavonoids with a terpenyl side chain.<sup>2-3</sup> The presence of these terpenyl side chains enhances biological activities such as cytotoxic, antioxidant, and estrogenic properties.<sup>4-7</sup> Flemilineatins A (**1**) and B (**2**), two new flavanones, along with 6-isoprenyl eridiocytol (**3**), were isolated from *F. lineata* leaves, and their cytotoxic effects on HeLa cells were reported here.

### Experimental

**General experimental procedures** – UV spectra were performed with a UV-VIS spectrophotometer (Shimadzu series 1800). The functional groups were recorded using an FT-IR spectrophotometer (Shimadzu IR Tracer 100). The mass molecule and the chemical formula were recorded using an ESI-TOF (Waters- LCT Premier XE).

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NMR spectra (JEOL JNM ECA-400) at 400 MHz for <sup>1</sup>H NMR and 100 MHz for <sup>13</sup>C NMR were measured using TMS as the internal standard and acetone-*d*<sub>6</sub> (δ<sub>H</sub> 2.04 and δ<sub>C</sub> 29.8, respectively) as reference standards. For column and radial chromatography, silica gel 60 and silica gel 60 PF<sub>254</sub> were employed, respectively. The optical rotation was measured with a polarimeter (Perkin Elmer Model 341).

**Plant material** – In February 2020, the fresh leaves of *F. lineata* were collected in Kemiren Village, Banyuwangi, East Java, Indonesia. A senior botanist (Ismail Rachman) from the Bogoriense Herbarium identified the plant. As a reference, a specimen (FL 20200218) was deposited.

**Extraction and isolation** – The dried leaves of *F. lineata* (8.1 kg) were extracted for a week with 90% methanol and then successively partitioned with *n*-hexane and ethyl acetate. The ethyl acetate extract (98 g) was separated on silica gel by column chromatography, using an *n*-hexane-ethyl acetate gradient (from 19:1 to 7:3 v/v) to yield three fractions A – C. Sephadex LH-20 column chromatography, eluting with methanol, divided fraction B into two subfractions, B<sub>1</sub> – B<sub>2</sub>. Compounds **1** (28 mg), **2** (9 mg) and **3** (18 mg) were obtained from fraction B<sub>1</sub> after separation by silica gel radial chromatography, eluting with *n*-hexane-chloroform gradient (from 19:1 to 3:7 v/v).

**Flemilineatin A (1)** – yellowish oil, [α]<sub>D</sub><sup>20</sup> = –3.2° (c 0.05, MeOH); UV (MeOH) λ<sub>max</sub> (log ε) 224 (4.52); 292 (4.17) and 343 nm (3.52). Table 1 shows the NMR data of **1**. HRESIMS *m/z* [M+H]<sup>+</sup> calculated for C<sub>30</sub>H<sub>37</sub>O<sub>6</sub> *m/z* 493.2649, found 493.2643.

**Table 1.**  $^1\text{H}$  (400 MHz),  $^{13}\text{C}$  NMR (100 MHz) data of **1** and **2** in acetone- $d_6$ 

		<b>1</b>			<b>2</b>		
No. C	$\delta_{\text{H}}$ (mult, $J$ in Hz)	$\delta_{\text{C}}$	HMBC	$\delta_{\text{H}}$ (mult, $J$ in Hz)	$\delta_{\text{C}}$	HMBC	
2	5.66 ( <i>dd</i> , 13.0; 2.8)	77.1	C-1', C-2', C-6'	5.61 ( <i>dd</i> , 13.0; 2.9)	77.3	C-1', C-2', C-6'	
3	3.13 ( <i>dd</i> , 17.1; 13.0) 2.62 ( <i>dd</i> , 17.1; 3,0)	43.3	C-2, C-4	3.18 ( <i>dd</i> , 17.2; 13.0) 2.67 ( <i>dd</i> , 17.2; 2.9)	43.2	C-2, C-4	
4	-	197.7	-	-	198.0	-	
4a	-	103.0	-	-	103.7	-	
5	-	162.2	-	-	165.0	-	
6	-	108.9	-	6.01 ( <i>d</i> , 2.2)	94.5	C-4a, C-7, C-8	
7	-	164.6	-	-	168.7	-	
8	6.00 ( <i>s</i> )	95.2	C-4a, C-6, C-7, C-8a	6.02 ( <i>d</i> , 2.2)	95.4	C-4a, C-6, C-7, C-8a	
8a	-	162.1	-	-	-	-	
9	3.24 ( <i>d</i> , 7.1)	21.6	C-5, C-6, C-7, C-10, C-11	-	-	-	
10	5.22 ( <i>t</i> , 7.3)	123.5	C-12, C-13	-	-	-	
11	-	131.5	-	-	-	-	
12	1.69 ( <i>s</i> )	25.7	C-10, C-11, C-13	-	-	-	
13	1.62 ( <i>s</i> )	17,8	C-10, C-11, C-12	-	-	-	
1'	-	129.4	-	-	129.2	-	
2'	-	125.8	-	-	125.8	-	
3'	-	143.9	-	-	144.0	-	
4'	-	144.1	-	-	144.1	-	
5'	-	127.0	-	-	127.0	-	
6'	6.91 ( <i>s</i> )	119.6	C-2, C-2', C-4', C-12'	6.92 ( <i>s</i> )	119.6	C-2, C-1', C-4', C-1'''	
7'	3.48 ( <i>d</i> , 6.9)	25.3	C-2', C-3', C-8'	3.49 ( <i>d</i> , 6.7)	25.3	C-3', C-7', C-8'	
8'	5.11 ( <i>t</i> , 6.6)	124.3	C-10', C-11'	5.12 ( <i>t</i> , 6.7)	124.3	C-10', C-11'	
9'	-	131.2	-	-	132.8	-	
10'	1.65 ( <i>s</i> )	25.8	C-8', C-9', C-11'	1.69 ( <i>s</i> )	17,8	C-8', C-9', C-11'	
11'	1.74 ( <i>s</i> )	17.8	C-8', C-9', C-10'	1.63 ( <i>s</i> )	25.7	C-8', C-9', C-10'	
12'	3.35 ( <i>d</i> , 7.2)	29.1	C-5', C-6', C-13', C-14'	3.35 ( <i>d</i> , 7.2)	29.1	C-4', C-13', C-14'	
13'	5.32 ( <i>t</i> , 7.3)	123.3	C-15', C-16'	5.32 ( <i>t</i> , 7.3)	123.3	C-15', C-16'	
14'	-	132.8	-	-	131.5	-	
15'	1,69 ( <i>s</i> )	25.8	C-13', C-14', C-16'	1.70 ( <i>s</i> )	17.9	C-13', C-14', C-16'	
16'	1.63 ( <i>s</i> )	18.0	C-13', C-14', C-15'	1.64 ( <i>s</i> )	25.8	C-13', C-14', C-15'	
3'-OH	-	-	-	7.33 ( <i>s</i> )	-	C-2', C-3'	
4'-OH	-	-	-	7.52 ( <i>s</i> )	-	C-4', C-5'	
5-OH	12.47 ( <i>s</i> )	-	C-4a, C-5, C-6	12.15 ( <i>s</i> )	-	C-4a, C-5, C-6	
7-OCH <sub>3</sub>	-	-	-	3.83 ( <i>s</i> )	56.2	C-7	

**Flemilineatin B (2)** – yellowish oil,  $[\alpha]_{\text{D}}^{20} = -3.1^\circ$  ( $c$  0.05, MeOH): UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 226 (4.50); 289 (4.20) and 341 nm (3.51). Table 1 shows the NMR data of **2**. HRESIMS  $m/z$   $[\text{M}+\text{H}]^+$  calculated for  $\text{C}_{26}\text{H}_{31}\text{O}_6$   $m/z$  439.2121, found 439.2112.

**6-Isoprenyl eridiol (3)** – yellowish oil, UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 226 (4.34); 295 (4.27) and 339 nm (3.45). The NMR data of **3** have been compared with the literature data.<sup>8</sup>

**Cytotoxicity assay** – In 96-well plates, HeLa cells were cultivated at a density of  $3 \times 10^4$  cells/cm<sup>3</sup>. For

growth, the cells were incubated at 37 °C for 24 hours. The isolates (**1** – **3**) were added to each well in triplicate with varying concentrations (100, 30, 10, 3, 1, 0.3, and 0.1  $\mu\text{M}$ ) and incubated at 37 °C for 48 hours. After incubation, the MTT reagent was applied to the culture cells and allowed to sit for four hours. A microplate reader spectrometer set to  $\lambda$  540 nm was used to measure the inhibition of cells by the isolates (**1** – **3**) was recorded.<sup>9</sup> Regression analysis was used to get the IC<sub>50</sub> values of the isolates (**1** – **3**).

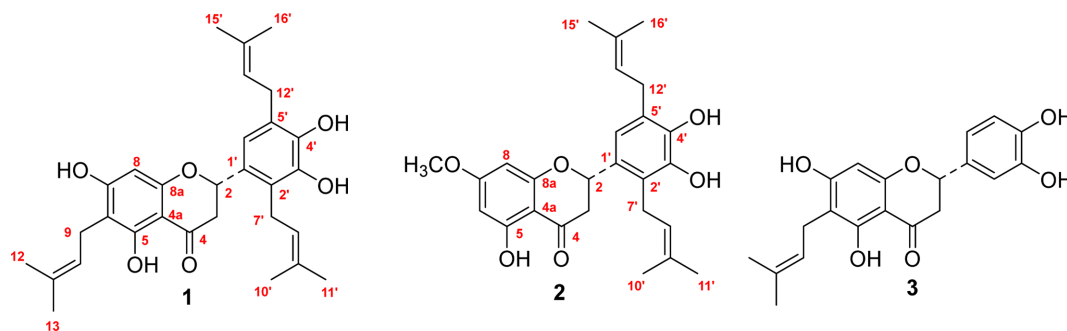


Fig. 1. Flavanones from *F. lineata* leaves.

## Results and discussion

Flemilineatin A (**1**) was isolated as a yellowish oil. The HRESIMS revealed the  $[M+H]^+$  ion at  $m/z$  493.2643 (calcd 493.2649), corresponding to the chemical formula  $C_{30}H_{37}O_6$ . The UV spectrum in MeOH ( $\lambda_{max}$  224, 292, and 343 nm) revealed the presence of a flavanone core.<sup>12</sup> The NMR data revealed the presence of an oxymethine proton [ $\delta_H$  5.66 (1H, *dd*,  $J=13.0, 3.0$  Hz, H-2),  $\delta_C$  77.1 (C-2)], distinct methylene protons [ $\delta_H$  3.13 (1H, *dd*,  $J=17.1, 13.0$  Hz, H-3<sub>ax</sub>), 2.62 (1H, *dd*,  $J=17.1, 3.0$  Hz, H-3<sub>eq</sub>),  $\delta_C$  43.3 (C-3)], and a conjugated carbonyl at  $\delta_C$  197.7 (C-4), which are characteristics for flavanone moiety.<sup>13</sup> Compound **1** also featured two protons of two aromatic units at  $\delta_H$  6.00 (1H, *s*, H-8) for ring A,  $\delta_H$  6.91 (1H, *s*, H-6') for ring B, and a hydroxy proton at  $\delta_H$  12.47 (1H, *s*, 5-OH). In addition, three isoprenyl protons were visible, including three olefinic protons [ $\delta_H$  5.32 (1H, *t*,  $J=7.3$  Hz, H-13'), 5.22 (1H, *t*,  $J=7.3$  Hz, H-10), 5.11 (1H, *t*,  $J=6.6$  Hz, H-8')], three methylene protons [ $\delta_H$  3.48 (2H, *d*,  $J=6.9$  Hz, H-7'), 3.35 (2H, *d*,  $J=7.2$  Hz, H-12'), 3.24 (2H, *d*,  $J=7.1$  Hz, H-9)], and six methyl protons [ $\delta_H$  1.74 (3H, *s*, H-11'), 1.69 (6H, *s*, H-12/H-15'), 1.65 (3H, *s*, H-10'), 1.63 (3H, *s*, H-16'), 1.62 (3H, *s*, H-13)]. The  $^{13}C$  NMR spectrum of flemilineatin A showed the signal of 28 carbons in the structure, including five oxyaryl carbons [ $\delta_C$  164.6, 162.2, 162.1, 144.1, 143.9], indicating that the flavanone structure is an eriodictyol derivative. The isoprenyl groups in the flavanone structure were identified using the HMBC spectrum. The chelated proton at  $\delta_H$  12.47 (5-OH) correlated to an oxyaryl at  $\delta_C$  162.2 (C-5) and two carbon signals at  $\delta_C$  103.0 (C-4a), and 108.9 (C-6). A methylene proton at  $\delta_H$  3.24 (H-9) correlated to C-5, C-6,  $\delta_C$  164.6 (C-7), a methine carbon at  $\delta_C$  123.5 (C-10), and a carbon signal at  $\delta_C$  131.5 (C-11), indicating an isoprenyl chain was located at C-6. Two methyl protons at  $\delta_H$  1.69 (H-12) and 1.62 (H-13) from the isoprenyl chain

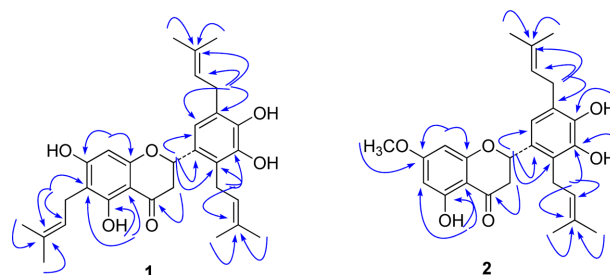


Fig. 2. HMBC of flemilineatins A (**1**) and B (**2**).

Table 2. Cytotoxic data of compounds **1** - **3**

Compounds	IC <sub>50</sub> (μM)	
	HeLa	MCF-7
Flemilineatin A ( <b>1</b> )	11.2 ± 0.3	36.4 ± 1.5
Flemilineatin B ( <b>2</b> )	>100	80.5 ± 1.2
6-Isoprenyl eriodictyol ( <b>3</b> )	>100	>100
Doxorubicin	4.5 ± 0.1	5.0 ± 0.2

correlated to C-10 and C-11 in favor of isoprenyl bound at C-6. An aromatic proton at  $\delta_H$  6.00 (H-8) correlated to C-4a, C-6, C-7, and oxyaryl at  $\delta_C$  162.1 (C-8a), was supported the location of an isoprenyl at C-6. An aromatic proton (ring B) at  $\delta_H$  6.91 (H-6') correlated to C-2, an oxyaryl at  $\delta_C$  144.1 (C-4'), a carbon signal at  $\delta_C$  103.0 (C-2'), and methylene at  $\delta_C$  29.1 (C-12'), indicating two isoprenyl chains were located at C-2' and C-5' in the ring B of eriodictyol structure. A methylene proton of 2-methyl-2-butenyl at  $\delta_H$  3.48 (H-7') correlated to an oxyaryl carbon at  $\delta_C$  143.9 (C-3'), a methine carbon at  $\delta_C$  124.3 (C-8'), and a carbon signal at  $\delta_C$  131.2 (C-9'), indicating that isoprenyl chain attached at C-2'. The long-range correlation between two methyl protons [ $\delta_H$  1.65 (H-10'), 1.74 (H-11')] to C-8', C-9', and an olefinic proton at  $\delta_H$  5.11 (H-8') to signals  $\delta_C$  25.8 (C-10') and  $\delta_C$  17.8 (C-11'), showing the isoprenyl located at C-2'. The methylene carbon at  $\delta_C$  29.1 (C-12') in the HMQC spectrum

exhibits direct connectivity with the proton signal at  $\delta_{\text{H}}$  3.35. The methylene proton at  $\delta_{\text{H}}$  3.35 (H-12') linked to C-4', two methine carbons [ $\delta_{\text{C}}$  119.6 (C-6'), 123.3 (C-13')], and two carbon signals [ $\delta_{\text{C}}$  127.0 (C-5'), 132.8 (C-14')], indicating that an isoprenyl located at C-5' (ring B). The stereochemistry of flemilineatin A at C-2 was determined to be *S* due to the negative specific rotation.<sup>12</sup>  
<sup>13</sup> In summary, the structure of flemilineatin A was determined to be (*S*)-6,2',5'-triosoprenyl-5,7,3',4'-tetrahydroxyflavanone.

Flemilineatin B (**2**) was isolated as a yellowish oil. The HRESIMS exhibited the  $[M+H]^+$  ion at  $m/z$  439.2112 (calcd 439.2121), consistent with the molecular formula  $C_{26}H_{31}O_6$ . The  $^1\text{H}$  and  $^{13}\text{C}$  NMR data of flemilineatin B were very similar to those of compound **1**, particularly at ring C [ $\delta_{\text{H}}$  5.61 (H-2), 3.18 (H-3<sub>ax</sub>), 2.67 (H-3<sub>eq</sub>),  $\delta_{\text{C}}$  77.3 (C-2), 43.2 (C-3), 198.0 (C-4)]. The presence of an aromatic group [ $\delta_{\text{H}}$  6.92 (H-6'),  $\delta_{\text{C}}$  119.6 (C-6')] and two isoprenyl chains [ $\delta_{\text{H}}$  5.32 (H-13'), 5.12 (H-7'), 3.49 (H-8'), 3.35 (H-12'), 1.70 (H-15'), 1.69 (H-10'), 1.64 (H-16'), 1.63 (H-11'),  $\delta_{\text{C}}$  132.8 (C-9'), 131.5 (C-14'), 124.3 (C-8'), 123.3 (C-13'), 29.1 (C-12'), 25.8 (C-16'), 25.7 (C-11'), 25.3 (C-7'), 17.9 (C-15'), 17.8 (C-10')] at ring B very similar to that of flemilineatin A. A pair of doublet protons ( $J=2.2$  Hz) at  $\delta_{\text{H}}$  6.01 (H-6), 6.02 (H-8), a chelated proton at  $\delta_{\text{H}}$  12.15 (5-OH), and a methoxy proton at  $\delta_{\text{H}}$  3.83 (7-OCH<sub>3</sub>) in the ring A were striking differences in the  $^1\text{H}$  NMR data of compound **2**. Signals for 28 carbons were identified in the  $^{13}\text{C}$  NMR spectrum. The HMBC spectrum, a chelated signal at  $\delta_{\text{H}}$  12.15 (5-OH) correlated to a methine at  $\delta_{\text{C}}$  94.5 (C-6), an oxyaryl at  $\delta_{\text{C}}$  165.0 (C-5), and a carbon signal at  $\delta_{\text{C}}$  103.7 (C-4a). The aromatic proton at  $\delta_{\text{H}}$  6.01 (H-6) correlated to C-4a, an oxyaryl at  $\delta_{\text{C}}$  164.6 (C-7), and a methine at  $\delta_{\text{C}}$  95.4 (C-8), while a methoxy group at  $\delta_{\text{H}}$  3.83 corresponded to C-7. The location of a methoxy group at to C-7 supports the long-range correlation between H-8 to C-4a, C-6, C-7, and an oxyaryl at  $\delta_{\text{C}}$  164.4 (C-8a). Due to the negative specific rotation, the stereochemistry of compound **2** at C-2 was also verified to be *S*.<sup>12-13</sup> As a result, the structure of flemilineatin B was determined to be (*S*)-2',5'-diiosoprenyl-5,3',4'-trihydroxy-7-methoxyflavanone.

The MTT technique was used to investigate the cytotoxicity of compounds **1–4** against HeLa cells. Negative controls included cells that had not been exposed to the active compound, while positive controls included doxorubicin.<sup>14-15</sup> Flemilineatin A (**1**) showed moderate activity with an IC<sub>50</sub> value of 11.2  $\mu\text{M}$ , and compounds (**2–3**) did not affect the HeLa cells. The presence of the isoprenyl

chain at C-6 of **1** increases activity than compound **2**, and the existing two 2-methyl-2-butenyl chains at C-2' and C-5' of **1** more than active with compound **3** against HeLa cells.<sup>15-16</sup>

In conclusion, two new flavanones, flemilineatins A and B (**1–2**), along with 6-isoprenyl eriodictyol (**3**), were isolated from the leaves of *F. lineata*. Compound **1** showed moderate activity against HeLa cells, and the other compounds (**2–3**) were inactive.

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## References

- (1) Heyne, K.; The useful Indonesian Plants, Research and Development Agency; Ministry of Forestry: Jakarta, **1987**, p 2114.
- (2) Tjahjandarie, T. S.; Tanjung, M.; Saputri, R. D.; Aldin, M. F.; Susanti, R. A.; Pertiwi, N. P.; Wibawa, R. S.; Halizah, I. N. *Phytochem. Lett.* **2021**, *44*, 78-81.
- (3) Rahman, M. M.; Sarker, D. S.; Byres, M.; Gray, A. I. *J. Nat. Prod.* **2004**, *67*, 402-406.
- (4) Fu, M.; Deng, D.; Huang, R.; Zhang, N.; Su, Z.; Qiu, S. X. *Nat. Prod. Res.* **2013**, *27*, 1237-1241.
- (5) Gumula, I.; Alao, J. P.; Ndiege, I. O.; Sunnerhagen, P.; Yenesew, A.; Erdélyi, M. *J. Nat. Prod.* **2014**, *77*, 2060-2067.
- (6) Li, L.; Deng, X.; Zhang, L.; Shu, P.; Qin, M. *Fitoterapia* **2011**, *82*, 615-619.
- (7) Xie, G.; Lin, B.; Qin, X.; Wang, G.; Wang, Q.; Yuan, J.; Li, C.; Qin, M. *Fitoterapia* **2015**, *104*, 97-101.
- (8) Marlina, E.; Astuti, W.; Kosala, K.; Hairani, R.; Tjahjandarie, T. S.; Tanjung, M. *Asian J. Chem.* **2018**, *30*, 795-798.
- (9) Saputri, R. D.; Tjahjandarie, T. S.; Tanjung, M. *Nat. Prod. Res.* **2021**, *35*, 1256-1261.
- (10) Tanjung, M.; Tjahjandarie, T. S.; Saputri, R. D.; Kurnia, B. D.; Rachman, M. F.; Syah, Y. M. *Nat. Prod. Res.* **2021**, *35*, 407-412.
- (11) Tjahjandarie, T. S.; Tanjung, M.; Saputri, R. D.; Rahayu, D. O.; Gunawan, A. N. I.; Aldin, M. F. *Nat. Prod. Res.* **2021**, *35*, 5637-5642.
- (12) Tanjung, M.; Tjahjandarie, T. S.; Sentosa, M. H. *Asian Pac. J. Trop. Dis.* **2013**, *3*, 401-404.
- (13) Syah, Y. M.; Hakim, E. H.; Achmad, S. A.; Hanafi, M.; Ghisalberty, E. L. *Nat. Prod. Commun.* **2009**, *4*, 63-67.
- (14) Tanjung, M.; Aldin, M. F.; Tjahjandarie, T. S.; Rahayu, D. O.; Gunawan, A. N. I.; Saputri, R. D. *Nat. Prod. Sci.* **2021**, *27*, 172-175.
- (15) Aldin, M. F.; Tjahjandarie, T. S.; Saputri, R. D.; Tanjung, M. *Nat. Prod. Sci.* **2021**, *27*, 45-48.
- (16) Saputri, R. D.; Tanjung, M.; Tjahjandarie, T. S. *Nat. Prod. Sci.* **2021**, *27*, 183-186.

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