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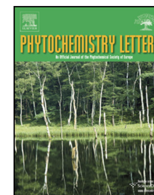

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Two new glycosides from *Duboscia macrocarpa* Bocq.

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ABSTRACT

Two new compounds namely dubosciasides A (**1**) and B (**2**) were isolated from stem bark of *Duboscia macrocarpa* Bocq. together with three known flavonoids. Their structures were determined by spectroscopic methods including one- and two-dimensional NMR, EIMS and HRESIMS. The new compounds were identified as 6-*C*-β-D-apiofuranosyl-(1 → 4)-β-D-galactopyranosylnaringetol (**1**) and 1-*O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-xylopyranosyl-3,4,5-trimethoxyphenol (**2**).
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1. Introduction

Duboscia is a small plant genus comprising two species belonging to the Malvaceae family. The genus was previously in the Tiliaceae family (Bocquillon and Baillon, 1866). It is now placed in the Malvaceae (Brink, 2007). One of the species, *Duboscia macrocarpa* Bocq. is a three which grows to 30 m and more with a fluted trunk. It occurs in Cameroon, Nigeria, and Democratic Republic of Congo. Little is known about the chemical constituents and biological properties of this plant as well as the other species. So far, there has been only one previous phytochemical investigation on *D. macrocarpa* (Wafo et al., 2010) which reported the isolation and structure elucidation of a new potent α-glucosidase inhibitor, duboscic acid, with a unique carbon backbone from the trunk wood. It is the first member of a new class of triterpenoid dubosane. As part of our continuing chemical studies of Cameroon plants (Kenmogne et al., 2006; Tabopda et al., 2012; Tchinda et al., 2014), we describe herein the chromatographic separation of dichloromethane-methanol extract of the stem barks of *D. macrocarpa* which resulted in the isolation of a phenolic and a flavanone glycosides together with three known flavonoids.

2. Results and discussion

The dichloromethane-methanol (1-1) extract of the stem barks of *D. macrocarpa* was subjected to a series of silica gel and Sephadex LH-20 chromatography columns to give two new glycosidic compounds (**1** and **2**) and three known flavonoids.

Compound **1** was isolated as a white amorphous powder and gave a dark blue coloration with $FeCl_3$ reagent. Moreover, it showed a positive Shinoda test (Sahu et al., 2010) suggesting that **1** is a flavonoid. The HRESIMS showed the molecular ion peak at *m*/*z* 566.5059 (calcd. for $C_{26}H_{30}O_{14}$, 566.5068) consistent with the molecular formula $C_{26}H_{30}O_{14}$. The ^{1}H NMR spectrum showed the presence of a singlet aromatic proton at δ_H 5.96 (s, H-8), and an AA′BB′ aromatic spin system at δ_H 6.83 (*d*, *J* = 8.7 Hz, H-3′,5′), 7.31, (*dd*, *J* = 8.7, 2.1 Hz, H-2′,6′). This observation suggested a penta-substituted A-ring and hence the AA′BB′ aromatic spin system is located on B-ring. The characteristic aliphatic protons of a flavanone skeleton were also observed at δ_H 2.74, 3.12, and 5.35 with the corresponding carbon atoms at δ_C 42.4 (C-3) and 79.0 (C-2) respectively. The remaining signals in the ^{1}H and ^{13}C NMR spectrum corresponded to those of two sugar units, which showed two anomeric protons at δ_H 4.80 (*d*, *J* = 7.8 Hz, H-1″) and 5.14 (*d*, *J* = 3.6 Hz, H-1‴) and δ_C 73.7 and 109.9, respectively. The upfield value of the anomeric carbon at δ_C 73.7 suggested a *C*-glycosidic linkage. Acid hydrolysis of **1** afforded a free sugar

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moiety and a mixture of 6-C-galactopyranosyl naringetol and 8C-galactopyranosyl naringetol in which the 8C-galactopyranosyl naringetol represent about one third. The sugar was identified by comparison of HPLC data with those of authentic sample and the absolute configuration was determined by the measurement of optical rotation and retention time to D-apiose. The ring H- and C-atoms of the sugar moieties were assigned starting from the readily identifiable anomeric H-atoms by means of COSY, NOESY, HMQC, and HMBC experiments. Evaluation of spin-spin couplings and chemical shifts allowed the identification of one β-D-galactopyranosyl (Gal) and one β-D-apiofuranosyl (Api). In the HMBC spectrum, correlations were observed between anomeric proton at δ_H 5.14 (Api H-1) and carbon atom at δ_C 78.9 (Gal C-4) on one hand and anomeric proton at δ_H 4.80 (Gal H-1) and carbon atoms at δ_C 104.6 (Agly C-6), 163.0 (Agly C-5), and 167.1 (Agly C-7) on the other hand. The ^{1}H NMR spectrum of **1** showed the presence of a phenolic hydroxyl group as a singlet at δ_H 13.12 strongly chelated to the carbonyl function at C-4. Furthermore, this proton indicated an important HMBC correlation with carbon at δ_C 163.0 (Agly C-5). All natural (−)-flavanones have been shown to have *S*-chirality at C-2, using circular dichroism spectroscopy (by observing the Cotton effect), thus the absolute configuration at C-2 was assigned as 2*S* (Dewick, 1994). The structure of **1** was shown to be 6-*C*-*β*-D-apiofuranosyl-(1 → 4)-*β*-D-galactopyranosylnaringetol, named dubosciaside A.

Compound **2** was obtained as white amorphous solid and was suggested to have the molecular formula $C_{19}H_{28}O_{12}$ based on HRESIMS [*m/z* 462.4442 $[M]^+$ (calcd. for $C_{36}H_{58}O_{10}$, 462.4438)]. It exhibited an UV absorption maximum at λ_{max} 294 nm, which implied the presence of a benzene ring (reference). The IR spectrum showed the presence of hydroxyl group(s) (ν_{max} 3393 cm^{-1}) and aromatic ring(s) (ν_{max} 1602, 1495 cm^{-1}). The ^{1}H NMR spectrum of **2** (Table 1) showed the presence of one 1,3,4,5-tetra-substituted aromatic ring and two anomeric protons at δ_H 4.72 (*d*, *J* = 1.8 Hz) and 4.84 (*d*, *J* = 7.8 Hz). This observation was further supported by the presence of a two-proton singlet at δ_H 6.46 in the ^{1}H NMR spectrum and HMBC correlations between protons at δ_H 6.46 and carbons at δ_C 153.4 (C-3, C-5), 133.4 (C-4), and 154.5 (C-1).The ^{1}H and ^{13}C NMR data of **2** (Table 1) showed signals for three methoxy groups at δ_H 3.72 (3H, s, 4-OMe) and 3.83 (6H, s, 3-OMe and 5-OMe) with the corresponding carbon atoms at δ_C 59.9 and 55.4. In the HMBC spectrum, the methoxy protons gave cross-peak with the C-3, C-4, and C-5 at δ_C 133.4 (C-4) and 153.4 (C-3, C-5), indicating a methoxy substitution at these positions. In the same experiment, the sugar proton at δ_H 4.84 (*d*, *J* = 7.8 Hz, H-1′) correlated with the carbon atom at δ_C 154.5 of the aglycone moiety, whereas the other sugar proton at δ_H 4.72 (*d*, *J* = 1.8 Hz, H-1″) gave cross-peak with carbon atom at δ_C 76.5 (C-4′). Acid hydrolysis of **2** afforded the trimethoxyphenol and the free sugars. The sugars were identified to xylose and rhamnose by TLC comparison with authentic samples and their absolute configurations were determined by the measurement of optical rotation after separation by preparative TLC. From the anomeric proton, each proton and carbon of the xylosyl and rhamnosyl groups was assigned by detailed analysis of ^{1}H–^{1}H COSY, HMQC, HMBC, and NOESY spectra. The ^{1}H NMR spectrum showed signals of an anomeric proton (δ_H 4.72, *d*, *J* = 1.8 Hz) and a secondary methyl group (δ_H 1.22, *d*, *J* = 6.8 Hz), indicating the presence of an α-L-rhamnose moiety. A characteristic doublet signal at δ_H 4.84 with coupling constant of 7.8 Hz was assigned to the anomeric proton in a β-D-xylopyranose unit. The base peak at *m/z* 183 in the EI mass spectrum corresponds to the trimethoxyphenol cation $[C_9H_{11}O_4]^+$. The other abundant ion at *m/z* 302 is due to the loss of a rhamnose residue (146 amu) from the molecular peak ion. Hence, the structure of **2** was determined to be 1-*O*-L-rhamnopyranoside-(1 → 4)-β-D-xylopyranosyl-3,4,5-trimethoxy-phenol, named dubosciaside B (Fig. 1). Q4

3. Materials and methods

3.1. General experimental procedures

Optical rotations were measure on a JASCO DIP-360 digital polarimeter. UV spectrum was recorded with an U-2910 spectrophotometer. IR spectra were recorded on a Perkin–Elmer 1750 FTIR spectrometer. The NMR spectra were recorded on Bruker Avance

Table 1
^{1}H (300 MHz) and ^{13}C (75 MHz) spectral data of Compound **1** (MeOH-d_4).

Position	δ_C	δ_H, mult., *J* in Hz	HMBC (^{1}H–^{13}C)	COSY
2	79.0	5.35 (dd, 3.0, 12.6)		H-3, H-3′
3	42.4	2.74 (dd, 3.0, 17.1)	C-2, C-4	H-2, H-3
		3.12 (dd, 12.6, 17.1)		
4	196.3	–	–	–
5	163.0	–	–	–
6	104.6	–	–	–
7	167.1	–	–	–
8	95.4	5.96 (s)	C-10, C-4, C-5, C-7	–
9	162.8	–	–	–
10	101.6	–	–	–
1′	129.6	–	–	–
2′,6′	127.6	7.31 (d, 8.4)	C-2, C-2′, C-6′, C-4′	H-3′, H-5′
3′,5′	115.0	6.83 (d, 8.4)	C-3′, C-5′, C-1′, C-4′	H-2′, H-6′
4′	157.6	–	–	–
Gal				
1″	73.7	4.80 (d, 10.2)	C-2″, C-6, C-7, C-5	H-2″
2″	71.0	4.24		H-1″, H-3″
3″	77.2	3.61		H-2″, H-4″
4″	78.9	3.60		H-3″, H-6″
5″	79.8	3.53	C-6″	H-6″
6″	60.6	3.80–3.90		H-6″, H-5″
Api				
1‴	109.9	5.14 (d, 3.6)	C-4″, C-3‴, C-4‴	H-2‴
2‴	76.4	3.97 (d, 3.6)	C-1‴, C-5‴	H-1‴
3‴	78.8	–	–	–
4‴	73.6	3.85 (d, 9.6)	C-1‴, C-3‴, C-2‴	H-4‴
		4.19 (d, 9.6)		
5‴	63.4	3.59 (s)	C-3‴, C-2‴, C-4‴	–

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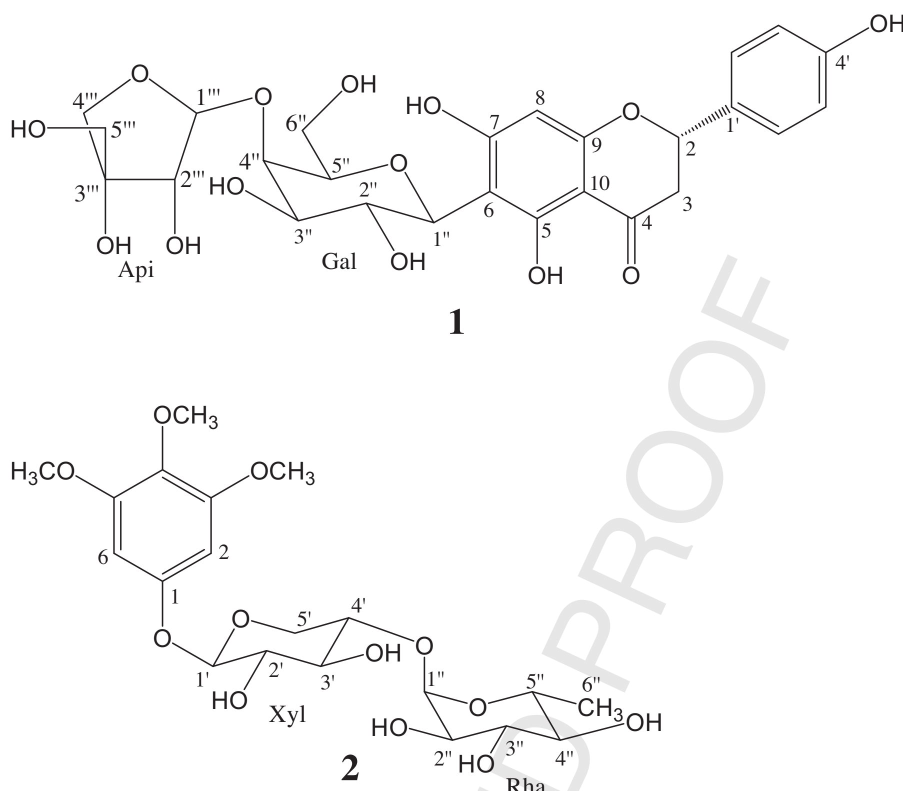

Fig. 1. Structures of compounds **1** and **2**.

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128 spectrometer (^{1}H resonance frequency of 300 MHz; ^{13}C resonance 129 frequency of 75 MHz). HRESIMS were run at 70 eV on a GCT 130 Premier MICROMASS instrument. EIMS were recorded at 70 eV 131 using VG70-SEQ. Precoated plates of silica gel 60 F_{254} and silica gel 132 60 (Merck; Darmstadt, Germany) were used for analytical and 133 preparative purposes, respectively. Detection of the spots was 134 achieved with a UV lamp at 254 nm and by spraying with 50% 135 H_2SO_4, followed by heating. Column chromatography (CC) was 136 carried out on Merck Silica gel (63–200 mesh and 40–63 mesh) 137 (Merk; Darmstadt, Germany) and Sephadex LH-20 (GE Healthcare, 138 Biosciences AB, Uppsala).

139 *3.2. Plant material*

140 The stem barks of *D. macrocarpa* Bocq. (Malvaceae) were 141 collected in Yaounde (Central Region of Cameroon) in June 2004 142 and identified by Dr. NoleTsabang, ethnobotanist at the Institute of 143 Medical Research and Medicinal Plants Studies (IMPM), Cameroon. 144 A voucher specimen No. W.C.S. 3642a/12543/Ya was deposited at 145 the National Herbarium of Yaounde.

146 *3.3. Extraction and isolation*

147 The dried and ground stem barks of *D. macrocarpa* (2.5 kg) were 148 macerated in CH_2Cl_2–MeOH (1:1) (3 mL × 7500 mL) duration at 149 room temperature. The extract (70 g) obtained after removal of 150 solvent under vacuum was separated by silica gel column 151 chromatography with EtOAc–MeOH gradient to give 5 fractions 152 (A–E). Fraction A (150 mg), eluted with $CHCl_3$–MeOH (97–3) was 153 rechromatographed on silica gel column using $CHCl_3$–MeOH 154 (99:1) to furnish a subfraction (63 mg) which was further 155 subjected to sephadex LH-20 column eluted with $CHCl_3$–MeOH 156 (70:30) to give fisetinidol (19 mg) (Mathisen et al., 2002). Repeated 157 column chromatography of fraction B (197 mg) with $CHCl_3$–MeOH 158 (95–5) followed by sephadex LH-20 column using $CHCl_3$–MeOH 159 (70–30) furnished catechin (51 mg) (Choudhary et al., 2013) and 160 epicatechin (38 mg) (Choudhary et al., 2013; Su et al., 2014). 161 Fraction C (253 mg) was applied on silica gel column chromatog- 162 raphy, eluting with $CHCl_3$–MeOH of increasing polarity to provide 163 a gumming subfraction (161 mg) which was repeatedly purified by 164 sephadex LH-20 using $CHCl_3$–MeOH (50:50) to yield catechin- 165 (4α→8)-epicatechin (72 mg) (Newman et al., 1987). Fraction D 166 (61 mg) was chromatographed twice on a silica gel column using 167 $CHCl_3$–MeOH (90:10) followed by gel filtration on sephadex LH-20 168 (MeOH) to give compound **2** (18 mg). Compound **1** (23 mg) was 169 obtained by subjecting fraction E (84 mg) (to repeated silica gel 170 ($CHCl_3$–MeOH 80:20) and sephadex LH-20 (MeOH) column 171 chromatography.

172 *3.4. Acid hydrolysis*

173 A soln. of **1** (10 mg) and **2** (10 mg) in 0.2 M HCl (dioxane–H_2O 174 1:1, 3 mL) was heated at 95 °C for 30 min under Argon. After 175 cooling, the mixture was neutralized by passage through an 176 Amberlite-IRA-93ZU (Organo, Tokyo, Japan) column and chroma- 177 tographed (Diaion HP-20, 40% MeOH followed by Me_2CO–EtOH 178 1:1) to give aglycone fractions (5.0 mg) and a sugar fraction 179 (3.3 mg). After the sugar fraction was passed through a Sep-Pak- 180 C_{18} cartridge (Waters, Milford, MA, USA; with 40% MeOH) and 181 Toyopak-IC-SP-M-cartridge (Tosoh; with 40% MeOH), it was 182 analyzed by HPLC (MeCN-H_2O 17: 3, flow rate, 0.9 mL min^{-1}; 183 detection, RI and OR): D-apiose (t_R 7.11, pos. OR), and D-galactose 184 (t_R 14.04, pos. OR) was detected in **1**, L-rhamnose (t_R 7.79, neg. OR) 185 and D-xylose (t_R 9.73, pos. OR) in **2**.

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Table 2
^{1}H (300 MHz) and ^{13}C (75 MHz) spectral data of Compound **2** in (MeOH-d_4).

Position	δ_C	δ_H, mult., J in Hz	HMBC (^{1}H–^{13}C)	COSY
1	154.5	–	–	–
2	95.1	6.46 (s)	C-2, C-4, C-1	–
3	153.4	–	–	–
4	133.4	–	–	–
5	153.4	–	–	–
6	95.1	6.46 (s)	C-2, C-4, C-1	–
1′	101.7	4.84 (d, 7.8)		H-2′
2′	73.5	3.45	C-3′	H-2′, H-1′
3′	75.5	3.57		H-2′, H-4′
4′	76.5	3.46		H-3′
5′	66.5	3.57 4.07 (d, 9.3)		H-4′, H-3′ H-5′
1″	100.7	4.72 (d, 1.8)	C-5′, C-5″, C-2″	H-3′
2″	70.7	3.61		
3″	71.0	3.82		
4″	72.7	3.33		
5″	68.4	3.63		
6″	16.5	1.22 (d, 6.8)		
3-OMe	55.4	3.83(s)	C-3	
5-OMe	55.4	3.83(s)	C-5	
4-OMe	59.9	3.72 (s)	C-4	

6-C-β-D-apiofuranoside-(1→4)-β-D-galactopyranosylnaringetol (**1**): white amorphous powder; $[\alpha]_D^{25}$-81.0 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 228 (7.2), 290 (7.0), 332 (6.4) nm; IR (KBr) ν_{max} 3427 (OH), 2918, 1722 (C=O), 1638 (C=C), 1334 cm^{-1}; ^{1}H- and ^{13}C-NMR (CD_3OD): see Table 1;.HRESIMS: m/z 566.5059 $[M]^+$ (calcd. for $C_{26}H_{30}O_{14}$, 566.5068); EI-MS: m/z (% int) 566 $[M]^+$ (13). 433 $[M\text{-}Api]^+$ (23), 313 (69), 119 (100); 1-O-L-rhamnopyranoside-(1→4)-β-D-xylopyranosyl-3,4,5-trimethoxyphenol (**2**): white amorphous solid; $[\alpha]_D^{25}+4.2$ (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 208 (4.4), 224 (3.9), 275 (3.1) nm; IR (KBr) ν_{max} 3396 (OH), 1602, 1495 (C=C) cm^{-1}; ^{1}H- and ^{13}C-NMR (CD_3OD): see Table 2; HRESIMS: m/z 462.4442 $[M]^+$ (calcd. for $C_{36}H_{58}O_{10}$, 462.4438); EI-MS: m/z (% int) 462 $[M]^+$ (47), 315 $[M\text{-}Rha]^+$ (41), 183 $[M\text{-}Rha\text{-}Xyl]^+$ (100), 447 $[M\text{-}15]^+$ (31);

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Agrawal (1989).

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References

Agrawal, P.K., 1989. Studies in Organic Chemistry, ^{13}C NMR of Flavonoids. Elsevier, Amsterdam, Oxford, New-York, Tokyo, pp. 455.
Bocquillon, H.T., Baillon, H.E., 1866. Adansonia 7, 50. Q8
Brink, M., 2007. In: Louppe, D., Oteng-Amoako, A.A., Brink, M. (Eds.), *Duboscia macrocarpa* Bocq. [Internet] Fiche de PROTA4U. PROTA (Plant Resources of Tropical Africa/Ressources végétales de l'Afrique tropicale), Wageningen, Pays Bas. http://www.prota4u.org/search.asp (accessed 4.11.13).
Choudhary, A., Mittal, A.K., Radhika, M., Tripathy, D., Chatterjee, A., Banerjee, U.C., Sindh, I.P., 2013. Two new stereoisomeric antioxidant triterpenes from *Potentilla fulgens*. Fitoterapia 91, 290–297.
Dewick, P.M., 1994. In: Harborne, J.B. (Ed.), The Flavonoids – Advances in Research Since 1986. Springer, New York, p. 117.
Kenmogne, M., Prost, E., Harakat, D., Jacquier, M.J., Frédérich, M., Sondengam, B.L., Zèches, M., Waffo-Téguo, P., 2006. Five labdane diterpenoids from the seeds of *Aframomum zambesiacum*. Phytochemistry 67, 433–438.
Mathisen, E., Diallo, D., Andersen, M., Malterud, K.E., 2002. Antioxidants from the bark of Burkea Africana, an African medicinal plant. Phytother. Res. 16, 148–153.
Newman, R.H., Porter, L.J., Foo, L.Y., et al., 1987. High-resolution ^{13}C NMR studies of proanthocyanidin polymers. Magn. Reson. Chem. 25, 118.
Sahu, V.K., Irchhaiya, R., Shashi, A., Gurjar, H., 2010. Phytochemical investigation and chromatographic evaluation of the ethanolic extract of whole plant extract of *Dendrophthoe falcata* (L.F.) Ettingsh. Int. J. Pharm. Sci. Res. 1, 39–45.
Su, D., Ti, H., Zhang, R., Zhang, M., Wei, Z., Deng, Y., Guo, J., 2014. Structural elucidation and cellular antioxidant activity evaluation of major antioxidant phenolics in lychee pulp. Food Chem. 158, 385–391.
Tabopda, T.K., Mitaine-Offer, A.C., Miyamoto, T., Tanaka, C., Mirjolet, J.F., Duchamp, O., Ngadjui, B.T., Lacaille-Dubois, M.A., 2012. Triterpenoidal saponins from *Hydrocotyle bonariensis*. Phytochemistry 73, 142–147.
Tchinda, A.T., Jansen, O., Nyemb, J.N., Tits, M., Dive, G., Angenot, L., Frédérich, M., 2014. Strychnobaillonine, an unsymmetrical bisindole alkaloid with an unprecedented skeleton from *Strychnos icaja* roots. J. Nat. Prod. 77, 1078–1082.
Wafo, P., Kamdem, R.S.T., Ali, Z., et al., 2010. Duboscic acid: a potent α-glucosidase inhibitor with an unprecedented triterpenoidal carbon skeleton from *Duboscia macrocarpa*. Org. Lett. 12 (24), 5760–5763.

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