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ALKALOIDS FROM *CASSYTHA FILIFORMIS*

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Key Word Index—*Cassytha filiformis*; Lauraceae; alkaloids; cathafiline; cathaformine.

Abstract—Cathafiline and cathaformine, two novel aporphinoid alkaloids possessing a *N*-(methoxycarbonyl) group, along with six known alkaloids, actinodaphnine, cassythine, isoboldine, cassameridine, cassamedine and lysicamine, have been isolated and characterized from *Cassytha filiformis*. The structures of these compounds were elucidated by spectroscopic analyses and from chemical evidence. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Cassytha filiformis commonly known as 'Wu-ken-taso', is a twining parasitic herb which is widely distributed along the seashore of southern Taiwan [1]. It is reported to be useful medicinally for gonorrhoea, kidney ailments and as a diuretic [2]. Previous investigations have shown the parasitic genus *Cassytha* to be a rich source of aporphinoid alkaloids and they have been reported from *C. filiformis* [3–6], *C. pubescens* [7], *C. glabella* [7], *C. melantha* [8] and *C. racemosa* [9]. Moreover, the isolation of cassyfiline had been reported from the Taiwan species [3]. As part of our continuing search for bioactive compounds from Formosan plants, two new aporphinoids alkaloids, cathafiline (**1**) and cathaformine (**2**), along with six known compounds, actinodaphnine (**3**), cassythine (**4**), isoboldine (**5**), cassameridine (**6**), cassamedine (**7**) and lysicamine (**8**), have been obtained by systematic extraction from *C. filiformis*. We report herein the structural elucidation of the two novel alkaloids **1** and **2**, which are the second examples of aporphinoid alkaloids possessing a *N*-(methoxycarbonyl) group [10]. In addition to the new alkaloids, compound **5** was found for the first time in this species and compound **8** was also isolated for the first time from a member of the Lauraceae.

RESULTS AND DISCUSSION

Cathafiline (**1**) was obtained as a brown amorphous from chloroform. The high-resolution EI mass spectrum revealed a $[M]^+$ at *m/z* 369.1210 (calcd 369.1213), corresponding with the molecular formula $C_{20}H_{19}NO_6$. The UV spectrum showed intense absorption bands at λ 236, 286 and 305 nm, which suggested that it possessed a typical 1,2,9,10-tetrasubstituted aporphine skeleton [11]. The IR spectrum of **1** exhibited absorption bands at 3520, 1680, 1040 and 920 cm^{-1}, indicating hydroxyl, carbonyl and methylenedioxy groups, respectively. Further support for the existence of a hydroxyl group in **1** was provided by a bathochromic shift of UV absorption on addition of alkali and the formation of a monoacetate (**1a**). Except for the protons affected by the *N*-(methoxycarbonyl) function, the general features of the ^1H NMR spectrum of **1** paralleled those for actinodaphnine (**3**) [12]. The ^1H NMR spectrum showed one methoxyl singlet at δ 3.94 (3H, *s*), one broad hydroxyl singlet at δ 5.74 (1H, *br s*, the signal disappeared by addition of deuterium oxide) and three aromatic proton signals at δ 7.67 (1H, *s*), 6.83 (1H, *s*) and 6.55 (1H, *s*), out of which the former low-field position can be assigned to H-11, the last two for the H-8 and H-3 in an aporphine nucleus, respectively. In addition to a singlet due to a methoxycarbonyl group at δ 3.76 (3H, *s*) and two singlets due to methylenedioxy protons at δ 6.09 and 5.96 (each 1H, *d*, J = 1.6 Hz), this accounted for 12 protons. The other proton signals were at δ 4.82 (1H, *m*), 4.40 (1H, *m*) and 2.97–2.60 (5H, *m*) for seven aliphatic protons. Two significant down-field signals at δ 4.82 (1H, *m*) for H-6a and δ 4.40 (1H, *m*) for H-5a indicated an electron-withdrawing group bonded to the nitrogen atom. The complete assignments of the relative configuration of aliphatic and aromatic protons of **1** was established by ^1H–^1H COSY and ^1H–^1H NOESY (Fig. 1) experiments.

Significant correlation between H-3, H-4 and H-5, H-6a, H-7 and H-8, as well as C_{10}-OCH_3 and H-11, was observed in the ^1H–^1H COSY and NOESY spec-

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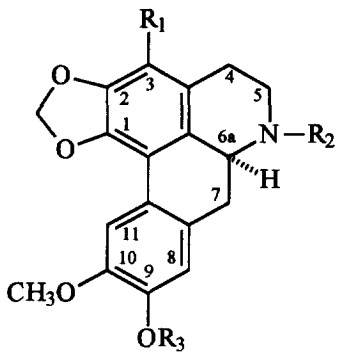

1. R_1=H, R_2=$COOCH_3$
2. R_1=OCH_3, R_2=$COOCH_3$, R_3=H
3. R_1=R_2=R_3=H

1a. R_1=H, R_2=$COOCH_3$, R_3=$COCH_3$
2a. R_1=OCH_3, R_2=$COOCH_3$, R_3=$COCH_3$
4. R_1=OCH_3, R_2=R_3=H

tra. In particular, the protons of the carboxyl methyl group at δ 3.76 did not correlate with any other protons. The ^{13}C NMR spectrum of **1**, which showed the chemical shift of a carbonyl carbon at δ 155.9, 12 aromatic carbon atoms between δ 146.6 and 106.7, a methylenedioxy carbon atom at δ 100.7, a methoxyl carbon atom at δ 56.1, as well as one carboxyl methyl carbon atom at δ 52.6, provided the elucidation of this structure. The *N*-carbonyl and aromatic methoxyl carbons in aporphines usually resonate at δ 161.0 and 56.0, respectively [13]; in compound **1** the two signals occurred at δ 155.9 and 52.6, respectively. From the up-field shift of the resonance of the carboxyl methyl carbon and, furthermore, on the basis of the chemical shifts of H-6a and H-5a in the ^1H NMR, the chemical shift of carbonyl carbon in the ^{13}C NMR and the absence of correlation with the methoxyl carbon group (δ 3.76) with any other proton in the NOESY spectrum, it was suggested that the methoxyl carbonyl group was attached to the nitrogen atom. Although the spectral data supported the structure **1** for cathafiline, the presence of a rare *N*-(methoxycarbonyl) function could not be established unequivocally. Chemical evidence from the preparation of a *N*-(methoxycarbonyl) derivative was thus undertaken. Treatment of actinodaphnine (**3**) with triethylamine and methyl chlorocarbonate gave a compound that had mp, UV, IR, TLC and NMR data identical to those of **1**. From the above discussion, cassafiline (**1**) is a novel alkaloid and should be represented as **1**.

Cathaformine (**2**) was obtained as a brown amorphous extract from chloroform. The high-resolution EI mass spectrum gave an $[M]^+$ at *m/z*, 399.1315 (calcd 399.1318), consistent with the molecular formula $C_{21}H_{21}NO_7$. The similarity in UV and IR spectral data between **1** and **2** suggested that they are analogues. The UV spectrum of **2** exhibited absorption bands at λ 239, 276 and 305 nm, revealing that it possessed a typical 1,2,3,9,10-pentasubstituted aprophine skeleton [11]. The IR spectrum exhibited absorption bands at ν_{max} 3400, 1670, 1040 and 920 cm^{-1}, indicating hydroxyl, carbonyl and methylenedioxy groups, respectively. A bathochromic shift of UV absorption on addition of alkali and the formation of a monoacetate derivative (**2a**) further supported the existence of a hydroxyl group in **2**. Except for the protons affected by the *N*-(methoxycarbonyl) function, the general features of the ^1H NMR spectrum of **2** also paralleled those for cassythine (**4**) [12]. Two methoxyl singlets were present at δ 4.00 (3H, *s*) and 3.93 (3H, *s*), assignable to C-3 and C-10, one broad hydroxyl singlet appeared at δ 5.66 (1H, *br s*, the signal disappeared by addition of deuterium oxide), and two aromatic singlets were also in evidence, with the more down-field, at δ 7.62, due to H-11, while the other at δ 6.82 was attributed to H-8, in accordance with the literature [12]. In addition to the signals of a methylenedioxy group, which was assigned to C-1 and C-2, appearing at δ 6.09 and 5.94 (each 1H, *d*, J = 1.6 Hz), and a singlet due to a methoxycarbonyl group at δ 3.76 (3H, *s*), this accounted for 14 protons. The other proton signals were at δ 4.79 (1H, *m*), 4.40 (1H, *m*) and 2.90–2.46 (5H, *m*) for seven aliphatic protons. Two significant down-field signals at δ 4.79 for H-6a and δ 4.40 for H-5a indicated an electron-withdrawing group bonded to the nitrogen atom. The relative configuration of protons of **2** was established by ^1H–^1H COSY and ^1H–^1H NOESY (Fig. 1) experiments.

Significant correlation between methylenedioxy and C_3-OCH_3, H-4 and H-5, H-6a, H-7 and H-8, as well as C_{10}-OCH_3 and H-11 was observed in the ^1H–^1H COSY and NOESY spectra. In particular, the protons of the carboxyl methyl group at δ 3.76 did not correlate with any other protons, the same as in **1**. The ^{13}C NMR spectrum of **2**, which showed the chemical shift of a carbonyl carbon at δ 155.9, 12 aromatic carbon atoms between δ 145.4 and δ 109.6, a methylenedioxy carbon atom at δ 100.8, two methoxyl carbon

atoms at δ 59.7 and 56.2, as well as one carboxyl methyl carbon atom at δ 52.6, supported the elucidation of **2**. As for **1**, on the basis of the chemical shifts of H-6a and H-5a in the ^{1}H NMR, the chemical shift of carbonyl carbon in the ^{13}C NMR and the absence of correlation of the methoxyl carbon group (δ 3.76) with any other proton in the NOESY spectrum, it was suggested that the methoxyl carbonyl group was attached to the nitrogen atom. Although the spectral data supported structure **2** for cathaformine, chemical evidence was also employed by the preparation of a *N*-(methoxycarbonyl) derivative of **4**. Treatment of cassythine (**4**) with triethylamine and methyl chlorocarbonate produced a compound that had mp, UV, IR, TLC and NMR data consistent with those of **2**. From the above results, cathaformine should be represented as **2**. Cathafiline (**1**) and cathaformine (**2**) are novel aporphinoid alkaloids possessing a *N*-(methoxycarbonyl) group reported for the second time in the literature [10].

The known compounds **3**–**8** were isolated and characterized by comparison of physical and spectral data (UV, IR and NMR) with those in the literature [12]. Compound **5** was found for the first time in this species and compound **8** was also isolated for the first time from the Lauraceae.

EXPERIMENTAL

General. Mp are uncorr. ^{1}H NMR spectra were recorded at 400 and 200 MHz, ^{13}C NMR spectra at 100 and 50 MHz, in $CDCl_3$ using TMS as int. standard. EIMS were obtained at 70 eV. Silica gel 60 (Macherey-Nagel, 230–400 mesh) was used for CC and precoated silica gel plates (Macherey-Nagel, SIL G-25 UV_{254}, 0.25 mm) were used for prep. TLC.

Plant material. Cassythia filiformis L. was collected from Pingtung-Hsien, Taiwan in September, 1994. A voucher specimen is deposited in the Graduate Institute of Natural Products, Kaohsiung Medical College, Kaohsiung, Taiwan, Republic of China.

Extraction and isolation of alkaloids. Fresh herb (10 kg) was extracted repeatedly with MeOH at room temp. The combined MeOH extracts were evapd and partitioned to yield $CHCl_3$ and aqueous extracts. The bases in the $CHCl_3$ soln were extracted with 3% HCl. The HCl soln was then basified with NH_4OH and extracted with $CHCl_3$. The $CHCl_3$ soln was dried (K_2CO_3) and evapd to leave a brownish viscous residue (41 g). This was chromatographed on a silica gel (1500 g, 40 × 6 cm) column using *n*-hexane, $CHCl_3$ and MeOH mixts of increasing polarity to yield 100 frs of 120 ml. These were further combined into six frs according to TLC composition. Fr. 3 (3.05 g) eluted with *n*-hexane–$CHCl_3$ (1:1) was further purified by silica gel CC using the same solvent system to obtain cathafiline (**1**) (18 mg) and cathaformine (**2**) (30 mg). Fr. 5 (7.26 g) eluted with MeOH–$CHCl_3$ (1:20) was further sepd and purified by silica gel CC and prep. TLC (MeOH–$CHCl_3$, 1:10), to give cassythine (**3**) (1.2 g), actinodaphnine (**4**) (150 mg) and isoboldine (**5**) (15 mg), respectively. The fr. eluting with MeOH–$CHCl_3$ (1:12) was further separated and purified by silica gel CC and prep. TLC (MeOH–$CHCl_3$, 1:8) to give cassameridine (**6**) (55 mg), cassamedine (**7**) (20 mg) and lysicamine (**8**) (40 mg), respectively.

Cathafiline (**1**). Brown amorphous (18 mg), mp 118–120°. $[\alpha]_D$ + 142.4° (*c* 0.14, $CHCl_3$). UV λ_{max}^{EtOH} nm: 236, 286, 305. IR ν_{max} (neat) cm^{-1}: 3520, 1680, 1040, 920. HREIMS: $[M]^+$ *m/z* 369.1210 (calcd for $C_{20}H_{19}NO_6$, 369.1213); EIMS *m/z*: 369 $[M]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.67 (1H, *s*, H-11), 6.83 (1H, *s*, H-8), 6.55 (1H, *s*, H-3), 6.09 and 5.96 (each 1H, *d*, *J* = 1.6 Hz, -O-C$\underline{H}_2$-O-), 5.74 (1H, *br*, -OH), 4.82 (1H, *m*, H-6a), 4.40 (1H, *m*, H-5a), 3.94 (3H, *s*, C_{10}-OC$\underline{H}_3$), 3.76 (3H, *s*, -N-CO-OC$\underline{H}_3$), 2.97 (2H, *m*, H-5b and H-7a), 2.81 (2H, *m*, H-7b and H-4a), 2.60 (1H, *m*, H-4b). ^{13}C NMR (100 MHz, $CDCl_3$): δ 155.9 (*s*, -N-$\underline{C}$O-OCH$_3$), 146.6 (*s*), 145.4 (*s*), 145.3 (*s*), 142.0 (*s*), 129.5 (*s*), 127.8 (*s*), 124.9 (*s*), 122.7 (*s*), 117.6 (*s*), 114.7 (*d*, C-8), 110.1 (*d*, C-11), 106.7 (*d*, C-3), 100.7 (*t*, -O-$\underline{C}$H$_2$-O-), 56.1 (q, C_{10}-O$\underline{C}$H$_3$), 52.6 (*q*, -N-CO-O$\underline{C}$H$_3$), 51.9 (*d*, C-6a), 39.1 (*t*, C-4), 33.2 (*t*, C-7), 30.3 (*t*, C-5).

Acetylation of **1** (**1a**). Cathafiline (**1**) (7 mg) was acetylated (Ac_2O-pyridine, overnight, room temp.)

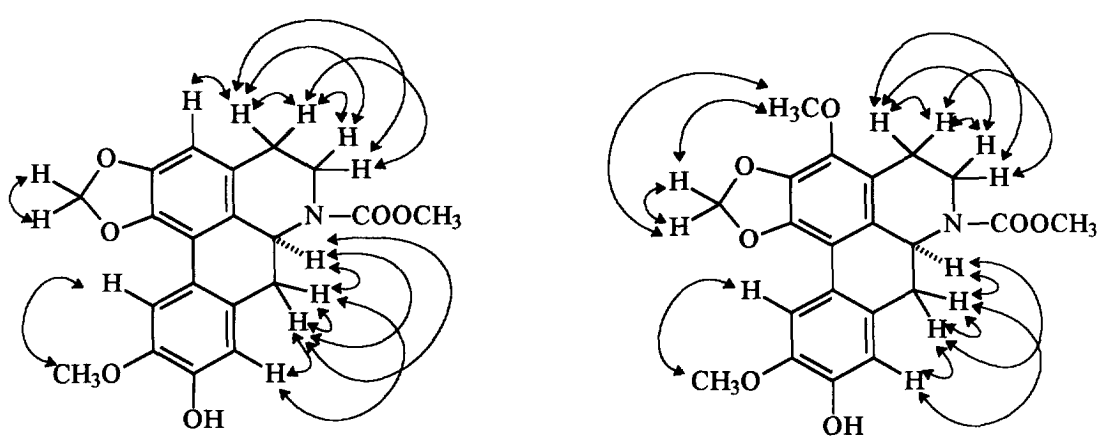

Fig. 1. NOESY spectral correlations of compounds **1** and **2**.

and the mixture partitioned between H_2O and $CHCl_3$. The $CHCl_3$ extract on chromatography afforded brown amorphous, *O*-acetyl cathafiline (**1a**). $[\alpha]_D$ +227.0° (*c* 0.2, $CHCl_3$). UV λ_{max}^{EtOH} nm: 239, 276, 305. IR ν_{max} (neat) cm^{-1}: 1740, 1680, 1040, 920. EIMS *m/z*: 411 $[M]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 7.78 (1H, *s*, H-11), 6.95 (1H, *s*, H-8), 6.59 (1H, *s*, H-3), 6.10 and 5.97 (each 1H, *d*, *J* = 1.6 Hz, -O-C<u>H</u>$_2$-O-), 4.84 (1H, *m*, H-6a), 4.43 (1H, *m*, H-5a), 3.88 (3H, *s*, C_{10}-OC<u>H</u>$_3$), 3.76 (3H, *s*, -N-CO-OC<u>H</u>$_3$), 2.96 (2H, *m*, H-5b and H-7a), 2.80 (2H, *m*, H-7b and H-4a), 2.64 (1H, *m*, H-4b), 2.33 (3H, *s*, -O-COC<u>H</u>$_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.4 (*s*, -O<u>C</u>OCH$_3$), 156.1 (*s*, -N-<u>C</u>O-OCH$_3$), 150.1 (*s*), 147.1 (*s*), 142.9 (*s*), 139.2 (*s*), 129.4 (*s*), 128.7 (*s*), 128.2 (*s*), 125.5 (*s*), 122.9 (*s*), 117.1 (*d*), 111.9 (*d*), 107.8 (*d*), 100.7 (*t*, -O-<u>C</u>H$_2$-O-), 56.1 (*q*, C_{10} -O<u>C</u>H$_3$), 52.6 (*q*, -N-CO-O<u>C</u>H$_3$), 51.6 (*d*, C-6a), 39.0 (*t*, C-4), 33.6 (*t*, C-7), 30.2 (*t*, C-5), 20.5 (*q*, -O-CO-<u>C</u>H$_3$).

Cathaformine (**2**). Brown amorphous (30 mg), mp 122–124°. $[\alpha]_D$ +116.4° (*c* 0.06, $CHCl_3$). UV λ_{max}^{EtOH} nm: 237, 286, 307. IR ν_{max} (neat) cm^{-1}: 3400, 1670, 1040, 920. HREIMS: $[M]^+$ *m/z* 399.1315 (calcd for $C_{21}H_{21}NO_7$, 399.1318); EIMS *m/z*: 399 $[M]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 7.62 (1H, *s*, H-11), 6.82 (1H, *s*, H-8), 6.09 and 5.94 (each 1H, *d*, *J* = 1.6 Hz, -O-C<u>H</u>$_2$-O-), 5.66 (1H, *br*, -OH), 4.79 (1H, *m*, H-6a), 4.40 (1H, *m*, H-5a), 4.00 (3H, *s*, C_3-OC<u>H</u>$_3$), 3.93 (3H, *s*, C_{10}-OC<u>H</u>$_3$), 3.76 (3H, *s*, -N-CO-OC<u>H</u>$_3$), 2.90 (2H, *m*, H-5b and H-7a), 2.62 (2H, *m*, H-7b and H-4a), 2.46 (1H, *m*, H-4b). ^{13}C NMR (100 MHz, $CDCl_3$): δ 155.8 (*s*, -N-<u>C</u>O-OCH$_3$), 145.4 (*s*), 144.7 (*s*), 143.4 (*s*), 139.1 (*s*), 135.4 (*s*), 128.9 (*s*), 126.0 (*s*), 122.9 (*s*), 120.5 (*s*), 114.6 (*d*), 111.2 (*d*), 109.6 (*d*), 100.8 (*t*, -O-<u>C</u>H$_2$-O-), 59.7 (*q*, C_3-O<u>C</u>H$_3$), 56.2 (*q*, C_{10}-O<u>C</u>H$_3$), 52.6 (*q*, -N-CO-O<u>C</u>H$_3$), 51.9 (*d*, C-6a), 38.8 (*t*, C-4), 34.1 (*t*, C-7), 23.9 (*t*, C-5).

Acetylation of 2 (**2a**). Cathaformine (**2**) (7 mg) was acetylated (Ac_2O-pyridine, overnight, room temp.) and the mixt. partitioned between H_2O and $CHCl_3$. The $CHCl_3$ extract on chromatography afforded brown amorphous *O*-acetyl cathaformine (**2a**). $[\alpha]_D$ +136.8° (*c* 0.08, $CHCl_3$). UV λ_{max}^{EtOH} nm: 240, 280, 302. IR ν_{max} (neat) cm^{-1}: 1720, 1660, 1040, 900. EIMS *m/z*: 441 $[M]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 7.72 (1H, *s*, H-11), 6.93 (1H, *s*, H-8), 6.10 and 5.93 (each 1H, *d*, *J* = 1.6 Hz, -O-C<u>H</u>$_2$-O-), 4.82 (1H, *m*, H-6a), 4.40 (1H, *m*, H-5a), 4.01 (3H, *s*, C_3-OC<u>H</u>$_3$), 3.87 (3H, *s*, C_{10}-OC<u>H</u>$_3$), 3.75 (3H, *s*, -N-CO-OC<u>H</u>$_3$), 2.95 (2H, *m*, H-5b and H-7a), 2.83 (2H, *m*, H-7b and H-4a), 2.44 (1H, *m*, H-4b), 2.33 (1H, *s*, -O-COC<u>H</u>$_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.5 (*s*, -O-<u>C</u>O-CH$_3$), 156.1 (*s*, -N-<u>C</u>O-OCH$_3$), 150.1 (*s*), 144.4 (*s*), 140.1 (*s*), 138.6 (*s*), 135.4 (*s*), 129.6 (*s*), 128.1 (*s*), 126.6 (*s*), 122.8 (*s*), 120.7 (*d*), 111.6 (*d*), 111.4 (*d*), 100.9 (*t*, -O-<u>C</u>H$_2$-O-), 59.6 (*q*, C_3-O<u>C</u>H$_3$), 56.1 (*q*, C_{10}-O<u>C</u>H$_3$), 52.6 (*q*, -N-CO-O<u>C</u>H$_3$), 51.5 (*d*, C-6a), 38.6 (*t*, C-4), 33.5 (*t*, C-7), 23.7 (*t*, C-5), 20.5 (*q*, -O-CO-<u>C</u>H$_3$).

Preparation of N-(*methoxycarbonyl*) *actinodaphnine* [*cathafiline* (**1**)]. Actinodaphnine (**3**) (20 mg) in dry CH_2Cl_2 (10 ml) was treated with Et_3N (3 μl), with stirring at 0° for 10 min, and then methyl chlorocarbonate (2 ml) was slowly added. While the reaction mixt. stirred for 10 min, H_2O was added to quench excess reagent. The mixt. was then partitioned with $CHCl_3$ and passed through a disposable pipette containing silica gel (230–400 mesh) and eluted with $CHCl_3$–MeOH gradually increasing the polarity to obtain a brown amorphous solid (8 mg, major product). The product was identified as **1** by direct comparison with the natural compound.

Preparation of N-(*methoxycarbonyl*) *cassythine* [*cassaformine* (**2**)]. Using the same method as described in the preparation of *N*-(methoxycarbonyl) actinodaphnine, cassythine (**4**) (20 mg) was treated with Et_3N and chlorocarbonate. A brown amorphous solid (7 mg) was obtained after separation and identified as **2** by direct comparison with the natural compound.

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