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Synthesis of Chiral Melatonin Salts of Dithiophosphoric Acids Using Monoterpenyl Alcohol

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Dithiophosphoric acids in the terms of (S)-(–)-menthol, (1*S*)-*endo*-(–)-borneol,(1*R*)-*endo*-(+)-fenchyl alcohol and (1*S*,2*S*,3*S*,5*R*)-(+)-isopinocampheol react with melatonin to form chiral *N*-acetyl 5-methoxytryptammonium dithiophosphates. The interations proceed *via* increase in the coordination of indole nitrogen of melatonin. The antibacterial and antifungal activity of *N*-acetyl 5-methoxytryptammonium dithiophosphate containing (+)-fenchyl substituent was established.

Keywords: Melatonin, Borneol, Fenchyl alcohol, Dithiophosphoric acids, Antimicrobial activity.

INTRODUCTION

It is realized that melatonin is combined in the pineal organ just as thymus, retina and others. Melatonin directs the phases of rest just as circadian musicality. It has been accounted for to be trap of free radicals as well as biological antioxidant agent [1]. Melatonin contains indole bicycle molecule, which plays a role as immunomodulator and neuroprotector [2]. It was reported that melatonin can control bacteria, viruses as well as parasitic infections. Melatonin appears to impacts on duplication of microorganisms and free radicals that can be remembered for the component of activities on germs [3]. The significance of melatonin is additionally identified with its security against sepsis. Pro-inflammatory mediators are affected by melatonin which increases its immunomodulating and antioxidant properties [1]. Melatonin diminishes intracellular substrates and in this manner shows excellent bactericidal properties [3]. Melatonin follows up on the resistant cells of the mucous film and the digestion of microbiota [4]. Ciprofloxacin based nephrotoxicity can be protected by melatone [5]. Ance is generally treated with melatonin [6].

In this way, melatonin itself displays the antimicrobial properties, however it′s manufactured phosphorylated derivatives have remained unknown until now. It is expected that phosphorylation of melatonin will prompt a synergistic impact in antimicrobial activities. Henceforth, modification of melatonin among organophosphorus compounds, dithiophosphoric acids were chosen. Most dithiophosphates are known to be less toxic to warm-blooded creatures [7]. No consideration has been paid for the synthesis of melatonin derivatives of phosphorus dithioacids. Herein, the results of the synthesis of *N*-acetyl 5-methoxytryptammonium dithiophosphates having pharmacophoric monoterpenyl functionalities are discussed.

EXPERIMENTAL

Dry argon was used for all reactions. Ethanol was dehydrated by distillation over sodium. Tetraphosphorus decasulfide (purity 99%), melatonin (purity 98%), (*S*)-(–)-menthol (purity 99%), (1*S*)-*endo*-(–)-borneol (purity 97%), (1*R*)-*endo*-(+)-fenchyl alcohol (purity 96%) and (1*S*,2*S*,3*S*,5*R*)-(+)-isopinocampheol (purity 98%) were procured from Sigma-Aldrich Co. (St. Louis, USA). Dithiophosphoric acid (**1a**) containing (*S*)-(–)-menthyl substituents was obtained using phosphorus pentasulfide [8]. Dithiophosphoric acid (**1b**) with (–)-borneolyl substituents was also prepared on the basis of phosphorus pentasulfide [9]. For the synthesis of dithiophosphoric acid

(**1c**) bearing (+)-fenchyl substituents, phosphorus pentasulfide and (1*R*)-*endo*-(+)-fenchyl alcohol were used [10]. Phosphorus pentasulfide and (1*S*,2*S*,3*S*,5*R*)-(+)-isopinocampheol were used to obtain dithiophosphoric acid (**1d**) [11].

Synthesis of compound 3a: A mixture of melatonin (**2**) (0.20 g, 0.86 mmol) and acid **1a** (0.35 g, 0.86 mmol) in 10 mL of anhydrous ethanol was vigorously stirred (20 ºC, 2 h) and then filtered. The filtrate obtained was evacuated (0.5 mm Hg) at 40 ºC for 1 h and (0.02 mm Hg) for 1 h to form *N*-acetyl-5-methoxytryptammonium *O,O*-di[(–)-(1*R*,2*S*,5*R*)-2-isopropyl-5-methylcyclohex-1-yl] dithiophosphate (**3a**) (0.48 g, 87%) as white solid, $[\alpha]_D^{20}$ –4.4 grad g^{-1} cm^2 (c = 1.03, acetone) (Tables 1-3).

Synthesis of *N*-acetyl 5-methoxytryptammonium *O,O*-di{*endo*-(1*S*)-1,7,7-trimethylbicycle[2.2.1]hept-2-yl-(–)}-dithiophosphate (**3b**) was obtained similarly as colourless solid (Tables 1-3).

Synthesis of *N*-acetyl 5-methoxytryptammonium *O,O*-di((1*R*)-*endo*-(+)-1,3,3-trimethylbicyclo[2.2.1]hept-2-yl)-dithiophosphate (**3c**) was obtained similarly as colourless amorphous semi-solid, $[\alpha]_D^{20}$ +2.2 grad g^{-1} cm^2 (c = 1.01, ethanol (Tables 1-3).

Synthesis of *N*-acetyl 5-methoxytryptammonium *O,O*-di((1*S*,2*S*,3*S*,5*R*)-(+)-2,6,6-trimethylbicyclo[3.1.1]hept-3-yl)-dithiophosphate (**3d**) was obtained similarly as white solid (Tables 1-3).

Biological assay: *in vitro* Antimicrobial properties of compound **3c** (1% DMSO) solutions were studied using bacterial and fungal cultures of *Staphylococcus aureus* ATCC 29213, *Bacillus cereus* and *Candida albicans* on the basis of Mueller-Hinton agar by gel diffusion test. Standard antimicrobial gel diffusion pocedures were performed.

Detection methods: A Bruker Avance-400 (161.9 MHz) and Bruker Avance (III) 400 spectrometers (Bruker BioSpin AG, Fällanden, Switzerland) were used for recording of the $^{31}P\{^1H\}$ and ^1H NMR spectra, respectively at ambient temperature. Ethanol was used to obtain $^{31}P\{^1H\}$ spectra. The ^1H NMR spectra were run using CD_3OD or acetone-d_6. A Bruker Tensor 27 infrared spectrophotometer (Bruker BioSpin AG, Fällanden, Switzerland) was operated to get Fourier transform IR spectra (KBr tablets or films). A Bruker Ultraflex mass spectrometer (Bruker Daltonik Gmbh, Bremen, Germany) with pulsed ultraviolet laser (λ = 337 nm) with a concentration of 1 wt.% in acetone were used for MALDI-TOF mass spectra. A Perkin-Elmer 341 polarimeter (Norwalk, CT, USA) (a pathlength = 55.2 mm, λ = 589 nm, sodium *D*-line) was practiced specific rotations $[\alpha]_D^{20}$ expressed as grad g^{-1} cm^2. An EuroEA3000 CHNS-O Analyzer (EuroVector S.p.A., Milano, Italy) (for composition of hydrogen, carbon, nitrogen and sulfur) and on a non-serial pyrolyzer (for phosphorus) were used.

RESULTS AND DISCUSSION

Interaction of *O,O*-di[(–)-(1*R*,2*S*,5*R*)-2-isopropyl-5-methylcyclohex-1-yl] dithiophosphoric acid (**1a**) with melatonin (**2**) was performed in anhydrous ethanol under mild conditions (20 ºC, 2 h) to give *N*-acetyl 5-methoxytryptammonium *O,O*-di[(–)-(1*R*,2*S*,5*R*)-2-isopropyl-5-methylcyclohex-1-yl]-dithiophosphate (**3a**) as white solid (**Scheme-I**).

TABLE-1
ANALYTICAL AND $^{31}P\{^1H\}$ NMR DATA OF COMPOUNDS OBTAINED

Comp.	Yield (%)	m.f.	Elemental analysis (%): Calc. (Found)					$^{31}P\{^1H\}$[a]
			C	H	N	P	S	δ (ppm)
3a	87	$C_{33}H_{55}N_2O_4PS_2$	62.04 (62.40)	8.68 (8.90)	4.38 (4.07)	4.85 (4.94)	10.04 (10.35)	93.3
3b	85	$C_{33}H_{51}N_2O_4PS_2$	62.43 (62.73)	8.10 (8.37)	4.41 (4.41)	4.88 (4.60)	10.10 (10.22)	107.2
3c	84	$C_{33}H_{51}N_2O_4PS_2$	62.43 (62.66)	8.10 (8.38)	4.41 (4.06)	4.88 (4.53)	10.10 (10.45)	96.5
3d	88	$C_{33}H_{51}N_2O_4PS_2$	62.43 (62.77)	8.10 (8.38)	4.41 (4.13)	4.88 (4.12)	10.10 (10.44)	102.6

[a]In ethanol

TABLE-2
SELECTED IR (ν, cm^{-1}) DATA OF COMPOUNDS OBTAINED

Compound	NHC=O	CNH	$C=C_{Ar}$	[(P)O–C]	P=S	P–S
3a (film)	1630	1586	1552, 1487	1041	669	548
3b (film)	1629	1587	1556, 1489	1041	666	568
3c (film)	1619	1585	1552, 1487	1040	674	564
3d (KBr)	1628	1586	1553, 1488	1039	670	569

TABLE-3
^1H NMR DATA OF COMPOUNDS OBTAINED

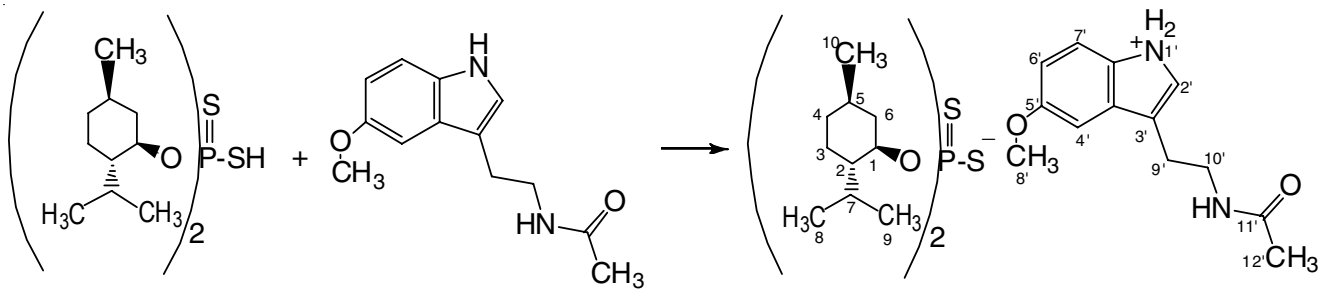
Compound	δ (ppm), *J* (Hz)
3a (CD_3OD)	0.79 d (6H, $C^{10}\underline{H}_3CH$, $^3J_{HH}$ 6.9), 0.90 d [12H, $(C^{8,9}\underline{H}_3)_2CH$, $^3J_{HH}$ 6.6 Hz], 0.91 d [12H, $(C^{8,9}\underline{H}_3)_2CH$, $^3J_{HH}$ 7.1], 1.13 m (2H, C^2H), 1.30 m [2H, $(CH_3)_2C^7\underline{H}$], 1.39 m (4H, C^3H_2), 1.41 m (2H, $CH_3C^5\underline{H}$), 1.62 m (4H, C^4H_2), 1.89 s (3H, $C^{12'}H_3C{=}O$), 2.28 m (4H, C^6H_2), 2.90 t (2H, $C^{9'}\underline{H}_2CH_2$, $^3J_{HH}$ 7.4), 3.48 t (2H, $CH_2C^{10'}\underline{H}_2N$, $^3J_{HH}$ 7.4), 3.82 s (3H, C^8H_3O-Ar), 4.13 m (2H, POC^1H), 6.75 d (1H, $C{=}C^{6'}H$, $^3J_{HH}$ 8.6), 7.12 s (1H, $C^{4'}H$, 1H, $C^{2'}H$), 7.26 d (1H, $C{=}C^{7'}H$, $^3J_{HH}$ 8.6), 9.90 m (2H, NH_2^+).
3b (acetone-d_6)	0.69 s (6H, $C^{10}H_3$), 0.73 s [12H, $(C^{8,9}H_3)_2C$], 1.43 m (4H, C^5H), 1.58 m (4H, C^6H_2), 1.73 s (3H, $C^{12'}H_3C{=}O$), 2.04 m (4H, C^3H_2, 2H, C^4H), 2.75 t (2H, $C^{9'}\underline{H}_2CH_2$, $^3J_{HH}$ 7.4), 3.33 t (2H, $CH_2C^{10'}\underline{H}_2$, $^3J_{HH}$ 7.4), 3.68 s (3H, C^8H_3O-Ar), 4.72 m (2H, POC^2H), 6.62 d (1H, $C{=}C^{6'}H$, $^3J_{HH}$ 8.9), 6.63 m (1H, $C^{2'}H$), 6.99 s (1H, $C^{4'}H$), 7.13 d (1H, $C{=}C^{7'}H$, $^3J_{HH}$ 8.7), 9.62 m (2H, NH_2^+).
3d (acetone-d_6)	0.80 s [6H, $(C^{8,9}H_3)_2C$], 0.96 d (6H, $C^{10}\underline{H}_3CH$, $^3J_{HH}$ 7.4), 1.08 s [6H, $(C^{8,9}H_3)_2C$], 1.61 m (4H, C^7H_2), 1.76 s (3H, $C^{12'}H_3C{=}O$), 1.91 m (2H, C^5H), 1.95 m (2H, C^1H), 2.21 m (2H, C^2H), 2.31 m (4H, C^4H_2), 2.76 t (2H, $C^{9'}\underline{H}_2CH_2$, $^3J_{HH}$ 7.4), 3.35 t (2H, $CH_2C^{10'}\underline{H}_2N$, $^3J_{HH}$ 7.4), 3.69 s (3H, C^8H_3O-Ar), 4.65 m (2H, P-OC^3H), 6.62 d (1H, $C{=}C^{6'}H$, $^3J_{HH}$ 8.6), 6.98 s (1H, C^4H), 7.01 s (1H, $C^{2'}H$), 7.13 d (1H, $C{=}C^{7'}H$, $^3J_{HH}$ 8.9), 9.65 m (2H, NH_2^+).

Compound **3a** is optically active ($[\alpha]_D^{20}$ –4.4 grad g^{-1} cm^2, c = 1.03, acetone) as well as acid **1a** ($[\alpha]_D^{22}$ –11.7 grad g^{-1} cm^2, c = 1.0, benzene) [8] and (*S*)-(–)-menthol ($[\alpha]_D^{20}$ –50 grad g^{-1} cm^2, c = 10, ethanol) [12]. In spite of the fact that these data were measured in different solvents, optical rotation sign does not change from chiral terpene alcohol through corresponding dithiophosphoric acid to its 5-methoxytryptammonium derivative. Compound **3a** containing 5-methoxytryptammonium cation gives a singlet at 93.3 ppm in the $^{31}P\{^1H\}$ NMR spectrum (ethanol) similarly other salts of dithiophosphoric acids [13]. A band at 1630 cm^{-1} is attributed to the NHC=O (amide I) stretching vibrations of 5-methoxytryptammonium cation in the FTIR spectrum of **3a** [14]. Similarly, ^1H NMR spectrum (acetone-d_6) of compound **3a** shows the presence of methyl groups in *O*-menthyl substituents. Two doublets at 0.90 ppm ($^3J_{HH}$ = 6.6 Hz) and 0.90 ppm ($^3J_{HH}$ = 7.1 Hz) were attributed to the resonances of protons of methyl groups of two fragments $(C^{8,9}\underline{H}_3)_2CH$. It should be emphasized that indole nitrogen atom is more basic than amide nitrogen. Therefore, indole nitrogen atom was protonated under the action of dithiophosphoric acids.

It was also found that dithiophosphoric acid (**1b**) with (–)-borneolyl substituents reacts with melatonin (**2**) in ethanol for 2 h at 20 ºC to form *N*-acetyl-5-methoxytryptammonium dithiophosphate (**3b**) (**Scheme-II**).

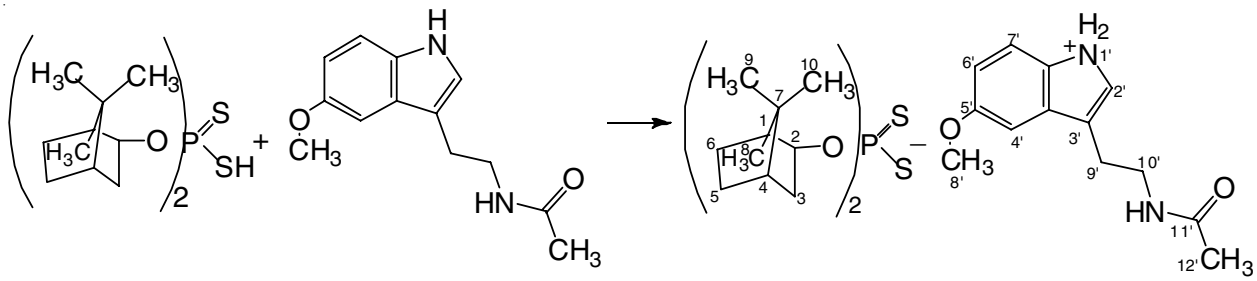
Compound **3b** revealed a singlet at 107.2 ppm in the $^{31}P\{^1H\}$ NMR spectrum (ethanol). Two bands at 1388 and 1370 cm^{-1} of compound **3b** were attributed to $\{\delta_s[(CH_3)_2C_{gem.}]\}$ deformation vibrations in its FTIR spectrum. Compound **3b** also showed a multiplet at 9.62 ppm due to protons of NH_2^+ group in the ^1H NMR spectrum.

It was decided to replace (*S*)-(–)-menthyl and (1*S*)-*endo*-(–)-borneolyl substituents with (1*R*)-*endo*-(+)-fenchyl ones in dithiophosphoric acids to synthesize new 5-methoxytryptammonium salt. So, a reaction of melatonin (**2**) with dithiophosphoric acid (**1c**) containing (+)-fenchyl substituents was carried out (**Scheme-III**). This reaction proceeded in ethanol for 2 h at 20 ºC and resulted in *N*-acetyl 5-methoxytryptammonium dithiophosphate (**3c**) in 84% yield. The optical rotation angle of compound **3c** was measured. As in the case of compound **3a** and acid **1a**, it is of interest to analyze similar values for acid **1c** ($[\alpha]_D^{22}$ +5.4 grad g^{-1} cm^2, c = 1.0, benzene) and salt compound **3c** ($[\alpha]_D^{20}$ +2.2 grad g^{-1} cm^2, c = 1.01, ethanol). Thus, sign of the values does not change. The $^{31}P\{^1H\}$ spectral signal of compound **3c** (96.5 ppm in ethanol) reavealed a significant change with respect to initial acid (**1c**) (87.6 ppm) [10]. The mass-spectrum of MALDI-TOF of **3c** reveals a mass peak of *m/e* 675.3 attributed to ion $[M + K + H]^+$ (calcd. molecular mass = 634.9).



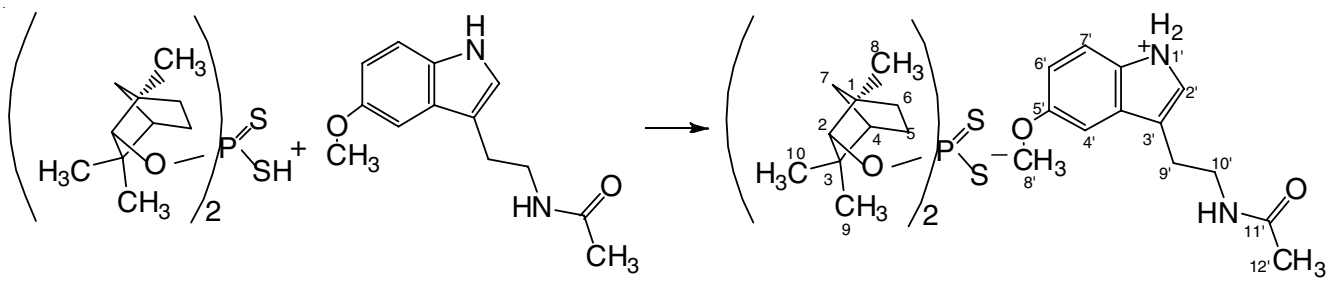
1a **2** **3a**

Scheme-I: Synthesis of salt **3a**



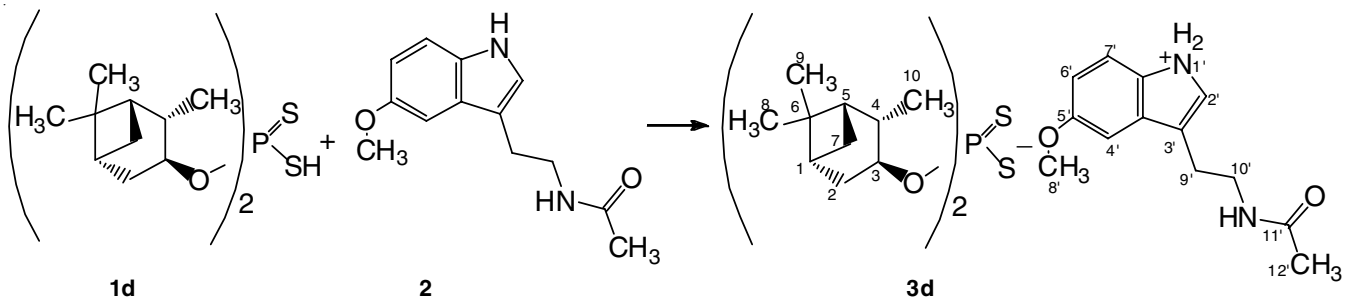
1b **2** **3b**

Scheme-II: Synthesis of salt **3b**



1c **2** **3c**

Scheme-III: Synthesis of salt **3c**



1d **2** **3d**

Scheme-IV: Synthesis of salt **3d**

The asymmetric carbon atoms were also varied in the terpenyl moiety and synthesized dithiophosphoric acid (**2d**) on the basis of (1*S*,2*S*,3*S*,5*R*)-(+)-isopinocampheol [11]. The reaction of compound **2d** with melatonin (**2**) in ethanol at 20 ºC for 1 h gives *N*-acetyl 5-methoxytryptammonium dithiophosphate (**3d**) (**Scheme-IV**).

Compound **3d** has a singlet in the same range (102.6 ppm) in $^{31}P\{^{1}H\}$ NMR spectrum as other 5-methoxytryptammonium salts. An intensive singlet at 1.76 ppm was attributed to protons of fragment $C^{12'}H_3C{=}O$ of compound **3d** in its ^{1}H NMR. In FTIR of compound **3d**, stretching vibrations of NHC=O (amide I) give band at 1628 cm^{-1}. As can be observed, by varying the O-monoterpenyl substituents in dithiophosphoric acids does not change the direction of the reaction.

Antimicrobial evaluation. The developed methods of synthesis of melatonin salts of dithiophosphoric acids was allowed to screen their antimicrobial activity. Among the obtained compounds, compound **3c** was chosen containing *O*-(1*R*)-*endo*-(+)-fenchyl substituent. Fenchyl alcohol and other monoterpenoids have been reported to possess remarkable antibiofilm activity against *Candida albicans* [15]. Compound **3c** (in 1% DMSO) was evaluated for its antibacterial activity (Table-4). Disinfectant Slayt was used in 1% concentration [16]. Compound **3c** shows a greater activity against *S. aureus* (growth inhibition zone of 28 mm) as compared to Slayt (19 mm). In the case of *B. cereus* and *C. albicans*, compound **3c** exhibited the same activity (14 and 13 mm, respectively) like as Slayt (12 and 14 mm, respectively).

TABLE-4
ANTIMICROBIAL ACTIVITY OF SALT **3c**[a,b]

Compound	*S. aureus*	*B. cereus*	*C. albicans*
3c	28	14	13
Slayt [16][c]	19	12	14

[a]In 1 % concentrations in DMSO; [b]Growth inhibition zone is expressed in mm; [c]In 1 % concentration.

Conclusion

O,O-Diterpenyl dithiophosphoric acids reacted with melatonin to form chiral *N*-acetyl 5-methoxytryptammonium dithiophosphates. In these reactions, indole nitrogen atom was protonated. The sign of optical rotation was preserved during the transition from the initial monoterpenyl alcohol *via* corresponding chiral *O,O*-diterpenyl dithiophosphoric acid to their melatonin derivatives.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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