R_f values and characteristic fluorescence showed the above mentioned toxins were not present in the cottonseed meals tested. When impurities were suspected of having a masking effect, further purification by column chromatography was carried out and the process was repeated.

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Oxidative Inactivation of Ethylenethiourea by Hypochlorite in Alkaline Medium

William D. Marshall* and Joginder Singh

The mechanism of hypochlorite mediated oxidation of ethylenethiourea (ETU) (I) to ethyleneurea (EU) (II) was investigated. Evidence is presented which suggests a sequential oxidation of ETU in aqueous base to a sulfenate, a sulfinate, a sulfonate, and finally to EU and sulfate. Although rapid and complete when using hypochlorite, the reaction is incomplete when using hydrogen peroxide, ETU being oxidized to the level of a sulfonate only. The synthesis of intermediates are detailed. Oxidation in alkaline medium is proposed as a technique for decontaminating surface residues of ETU on agricultural products.

Current concern regarding residues of ethylenebisdithiocarbamate (EBDC) fungicides centers on the possibility that these residues may be converted to ethylenethiourea (ETU) (I), which is a potent teratogen and tumorigen to mice and rats (Graham et al., 1975; Graham, 1973; Graham and Hansen, 1972; Khera, 1973; Innis et al., 1969). Under optimum laboratory conditions the conversion of EBDCs to ETU can be quite efficient (Marshall, 1977; Watts et al., 1974; Newsome and Laver, 1973). ETU has also been demonstrated to form from EBDC field residues during normal food processing techniques (Baron, 1976). During a search for chemical agents which would oxidatively inactivate these residues it was observed that aqueous hypochlorite reacted rapidly and completely with ETU and with nabam (disodium ethylenebisdithiocarbamate). As a prelude to a detailed study of the action of hypochlorite on EBDCs it was deemed necessary to study the oxidation of ETU in some depth. This paper details the mechanism of oxidation of ETU with hypochlorite and with hydrogen peroxide and describes the synthesis of intermediates on the oxidative pathway.

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EXPERIMENTAL SECTION

Materials. Jaffe's base [1-(2′-imidazolin-2′-yl)-2-imidazolidinethione] (III) was synthesized according to the method of Johnson and Edens (1942). Chromatography of the base on silica gel using methanol–water (4:1) as eluent resulted in a colorless crystalline product which was recrystallized from ethanol (mp 236–237 °C). ETU disulfide [bis(2-imidazolin-2-yl) disulfide] (IV) was prepared as the dihydrochloride according to the method of Freedman and Corwin (1949) (mp 231–234 °C). ETU (2-imidazolidinethione) and EU (2-imidazolidineone) were purchased from Fisher Scientific Co. ETU was recrys-

Table I. TLC Characteristics of Ethylenethiourea and Its Oxidized Derivatives

Compd	No.	R_f in solvent system: 9:1 Methanol/acetic acid	R_f in solvent system: Methanol	Detection using[a,b]: i	ii	iii	iv	v	vi	vii
ETU	I	0.65	0.57	+	Blue	+	+	Olive Green	Blue	+
EU	II	0.59	0.53	–	–	–	+++	–	–	–
Jaffe's Base	III	0.44	0.28	+	–	+	–	Red	Blue	+
ETU disulfide	IV	dec	dec	+	–	+	–	Red		+
ETU trioxide	V	0.62	0.57	–	–		+	Light brown	Purple	+
N,N-Dimethyl ETU trioxide	VI	0.49	0.37	+	–		+	Light brown	Purple	+
ETU dioxide	IX	0.17	0.13	+			+			+
N,N-Dimethyl ETU	X		0.50	–	Pale	–	+++	Light brown	Purple	

[a] i, Dragendorf reagent; ii, Grote's reagent; iii, iodine-sodium azide; iv, *N,N*-dimethylaminocinnamaldehyde; v, 2,6-dichloro-*N*-bromo-*p*-benzoquinoneimine; vi, ninhydrin; vii, fluorescence quench. [b] + = positive reaction; +++ = strong reaction; – = no reaction.

tallized from methanol–water (1:1) containing 5% hexane while EU was sublimed at 105 °C (10^{-3} mm) and recrystallized from methanol prior to use.

ETU trioxide (2-imidazolin-2-yl sulfonate) (V) was prepared by treating an ice-cold stirred suspension of finely ground ETU in carbon tetrachloride with an excess of 30% hydrogen peroxide. After stirring for 15 min the precipitate was collected and washed thoroughly with cold methanol. Two crystallizations from ethanol–water (1:1) furnished an analytical sample (mp 156–158 °C). Anal. Calcd for $C_3H_6N_2O_3S$: C, 24.00; H, 4.03; N, 18.66; S, 21.35. Found: C, 24.32; H, 4.23; N, 18.42; S, 21.38.

N,N-Dimethyl ETU trioxide (*N,N*-dimethyl-2-imidazolin-2-yl sulfonate) (VI) was prepared by reacting a vigorously stirred suspension of sulfonate V with excess ethereal diazomethane at room temperature. After an hour the supernatant was decanted, and the residues were carefully dried under a stream of nitrogen. Crystallization from water–methanol furnished an analytical sample as colorless needles (mp 237–239 °C). Anal. Calcd for $C_5H_{10}N_2O_3S$: C, 33.70; H, 5.66; N, 15.72; S, 17.99. Found: C, 33.92; H, 5.53; N, 15.41; S, 18.10. Sodium hypochlorite solutions were diluted from a commercial formulation containing not less than 6% active ingredient.

Method. Iodometric determinations of the amount of hypochlorite consumed were performed as follows: A measured excess of oxidant (to result in a 0.2% final solution) was added to a solution of substrate (0.3 mM) in 1% sodium bicarbonate (50 mL), which had previously been purged with nitrogen. The flasks were immediately capped, swirled, and the reaction allowed to proceed to completion at room temperature in the dark. Sulfuric acid (20 mL, 2 N) was added to quench the reaction followed by 300 mg of sodium bicarbonate in small portions. Finally, potassium iodide (2 g) was added and the flasks were restoppered, swirled, and set aside for 2 min. The solution was then titrated with standardized sodium thiosulfate (0.1 N solution) by adding either starch solution or Thyodene as indicator near the end point. When hydrogen peroxide was used as the oxidant, 5 drops of a neutralized 3.0% ammonium molybdate solution was added as a catalyst to ensure complete reaction between excess oxidant and potassium iodide. With iodine as oxidant the addition of potassium iodide was omitted and the titration was accomplished using either sodium thiosulfate (to the acidified reaction mixture) or arsenous oxide (to the reaction mixture containing 2 g of sodium bicarbonate) as titrant. In every case a parallel blank containing to substrate was run as a control.

Equipment. IR spectra were determined as KBr pellets using a Beckman IR-20A spectrophotometer. Proton NMR spectra were obtained in D_2O with ETU as internal standard on a Varian T-60 NMR spectrometer. EPR spectra were recorded at room temperature in 3 M ammonium hydroxide on a Varian 3 EPR spectrometer. The MS was determined on a Finnigan 3100 GC–MS coupled to a D6000 data acquisition system. Samples were determined either by direct inlet probe or on a 5 ft × 4 mm i.d. glass column packed with 3% OV-17 on 100–120 mesh Gas-Chrom Q.

Thin-Layer Chromatography. Compounds were spotted on silica gel plates (0.25 mm, Polygram-Macherey Nagel & Co.) containing a fluorescent indicator and developed in either 9:1 methanol–acetic acid or methanol. Products were visualized using fluorescence quenching, iodine vapors, or one of the following chromagenic sprays: Dragendorf reagent, Grote's reagent (1931), iodine–sodium azide, dimethylaminocinnamaldehyde in 3:1 ethanol–4 N hydrochloric acid, 2,6-dichloro-*N*-bromo-*p*-benzoquinoneimine in acetic acid or ninhydrin. The chromatographic behavior of products discussed in this paper is given in Table I.

RESULTS AND DISCUSSION

When sodium hypochlorite was reacted with a dilute aqueous solution of ETU, the only products detected by TLC were EU (II), elemental sulfur, and small but variable amounts of Jaffe's base (III). These products were identified by cochromatography with authentic standards and by isolation of the reaction products. After basifying the reaction mixture and exhaustively extracting with chloroform, addition of barium carbonate to the aqueous phase yielded a copious precipitate which was identified as barium sulfate by its infrared spectrum.

It has been demonstrated by Johnson and Edens (1941) that the reaction of alcoholic hypobromous acid or hypochlorous acid with ETU results in Jaffe's base and ethylenediamine. Jaffe's base is also the product of oxidation of ETU in alcohol with chloropicrin (Rây and Das, 1922). It was found that when ETU in acetonitrile is oxidized by dropwise addition of neutralized hypochlorite solution (which had been diluted with acetonitrile and

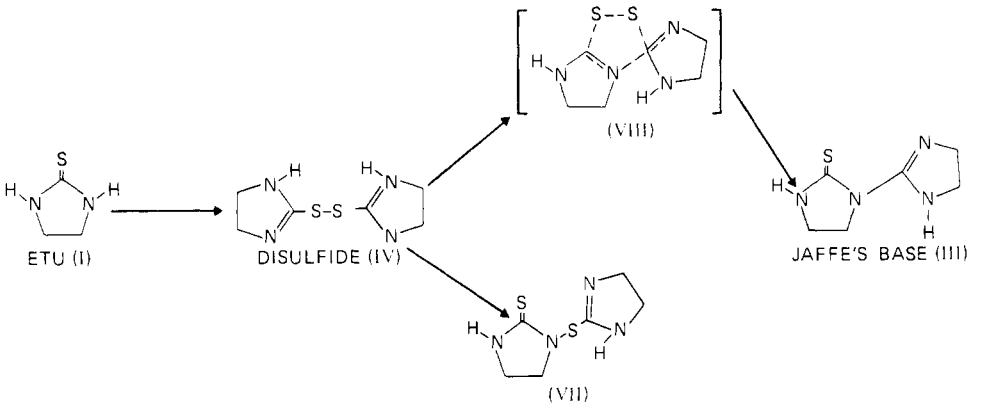

Figure 1. Formation of Jaffe's base from ETU.

Table II. Consumption of Oxidant by Ethylenethiourea and Oxidized Derivatives

Substrate	Oxidant	Medium[a]	Reaction time, min	Equiv oxidant[b] /equiv substrate
ETU	NaOCl	B	5	3.96
			20	3.98
			40	4.05
			60	3.99
ETU	H_2O_2	B	10	3.07
			20	2.99
			30	3.02
ETU	H_2O_2	A	15	0.99
			45	1.19
			90	1.73
			120	2.37
ETU	I_2	B	20	2.99
			40	3.04
			60	3.01
ETU	I_2	A	20	3.01
			40	2.96
ETU·O_3	NaOCl	B	30	0.96
			60	1.03

[a] B = 1% sodium bicarbonate solution, A = 0.1 N hydrochloric. [b] All values represent the average of duplicate or triplicate determinations.

filtered to remove precipitated sodium chloride), a precipitate is rapidly formed. The crude precipitate, when dissolved in the same solvent and subjected to GC–MS analysis, contained two products (M^+ 204 and 203) in addition to unreacted ETU. The major product gave a mass fragmentation pattern identical with authentic ETU disulfide (IV), and the latter minor product was assigned the tentative structure [1-(2′-imidazolin-2′-yl)-2-imidazolidinethione sulfide] (VII). Product IV is a diacid base whereas product VII is a monoacid base accounting for a molecular ion one mass unit less than the former. The mass fragmentation pattern of the rearranged product is quite different from that of disulfide IV. Although both products display intense molecular ions and M − 1 ions, product VII displays an intense ion at m/e 102 corresponding to ETU which is absent in the spectrum of the disulfide.

The disulfide dihydrochloride is unstable when stored as a solid (Freedman and Corwin, 1949). It is rapidly converted to Jaffe's base III and elemental sulfur on standing, during chromatography on silica gel or alumina or by boiling in water. Similar behavior was observed when ETU disulfide dihydroperiodide was boiled in water (Johnson and Edens, 1942). These observations suggest that minor yields of Jaffe's base III observed in the aqueous oxidation of ETU result from decomposition of initially formed ETU disulfide IV. A possible degradation scheme is diagrammed in Figure 1. Whether the rearranged product VII represents a genuine intermediate in the conversion of ETU to Jaffe's base remains unclear. It is possible that product VII results from on-column thermal isomerization of ETU disulfide IV in the gas chromatograph. *S*-Alkyl isothioureas are known to isomerize to the thermodynamically more stable *N*-alkyl thioureas under a variety of conditions. An alternate mechanism for the conversion of a substituted thioformamidine disulfide to the corresponding guanyl thiourea (IV to III) has been suggested by Lechner and Gubernator (1953) in which a cyclic transition state (VIII) is proposed.

To further elucidate the mechanism of oxidation in aqueous media ETU was treated with excess oxidant (0.2% solution), the reaction allowed to proceed to completion in the dark, and the excess of reagent was determined iodometrically by titration. In this study the consumption of hypochlorite, or hydrogen peroxide or iodine, in either acidic or basic medium was monitored. The results are reported in Table II. In acid (0.1 N hydrochloric) 1 equiv of hydrogen peroxide was rapidly consumed per equivalent of ETU; however, further oxidant was slowly consumed with time due to reaction of oxidant with decomposition products from the initially formed ETU disulfide IV. It is known that ETU oxidation in acid medium (Freedman and Corwin, 1949) is exactly analogous to the oxidation of thiourea which has been shown to result in formamidine disulfide in the same medium (Priestler and Berger, 1947).

When treated with aqueous iodine–potassium iodide (9:13) ETU in acidic or basic medium was found to consume 3 equiv of oxidant and resulted in the rapid formation of a fluocculent brown precipitate. The precipitate was very soluble in acetone and chloroform and when recrystallized from chloroform resulted in ETU

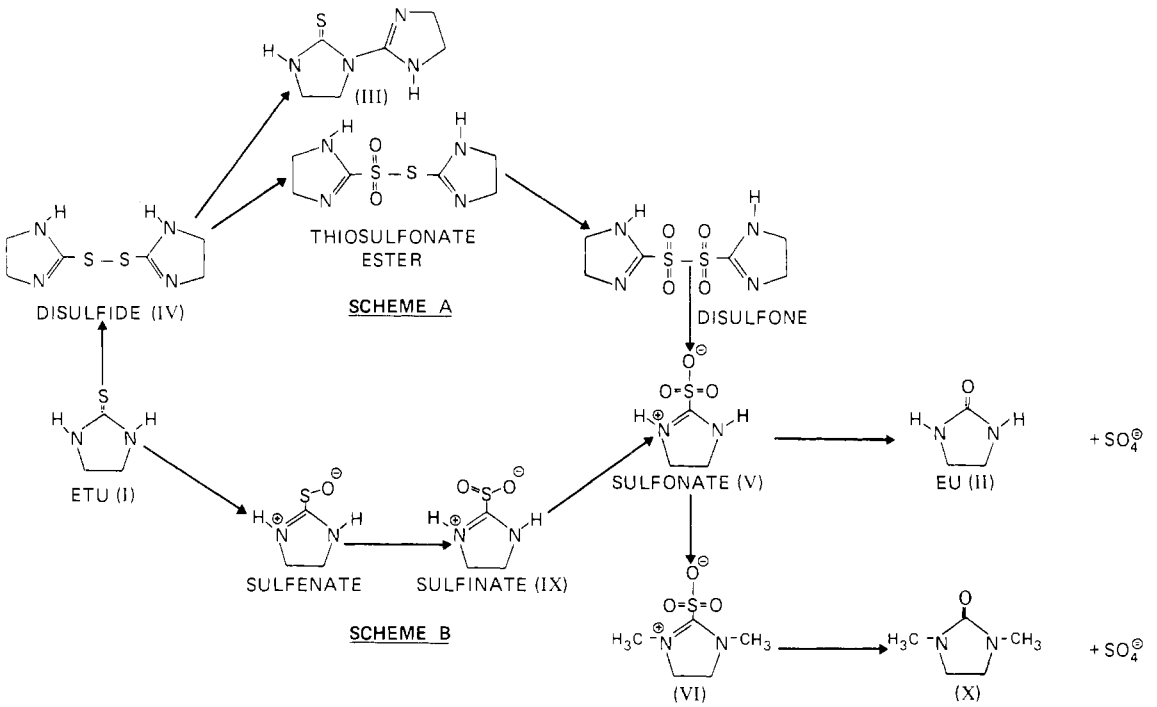

Figure 2. Potential oxidative pathways to ETU inactivation and derivatization of the oxidized intermediates.

disulfide dihydroperiodide (mp 119–120 °C), confirming the observations of Johnson and Edens (1942).

In basic medium (0.1 N bicarbonate), 4 equiv of hypochlorite were consumed per equivalent of ETU while only 3 equiv of hydrogen peroxide or iodine were consumed. In both acidic and basic medium EU remained unaffected by excess hypochlorite, hydrogen peroxide, or iodine. Two possible mechanisms which would account for these observations are detailed in Figure 2. In scheme A, ETU is sequentially oxidized to the disulfide IV, to a thiosulfonate ester, to a disulfone, to 2-imidazolin-2-yl sulfonate V, and finally to EU and sulfate. In scheme B, ETU is sequentially oxidized to a sulfenate, to a sulfinate, to sulfonate V, and finally to EU and sulfate. Oxidized derivatives of thiourea (analogues of postulated intermediates in scheme B) are readily prepared using hydrogen peroxide. Aminoiminomethane sulfinic acid (thiourea dioxide) results from oxidation of thiourea with 2 equiv of hydrogen peroxide (de Barry Barnett, 1910), an oxidation which may also be induced photolytically (Schneck and Wirth, 1953). Oxidation of the same substrate with an excess of hydrogen peroxide results in aminoiminomethane sulfonic acid (thiourea trioxide) (Shashoua, 1964). Oxidation of thiourea dioxide with iodine in basic solution results in urea and sulfate (Golovnya and Bolotova, 1961).

Treatment of ETU, as a finely ground suspension in an inert solvent, with 3 equiv of hydrogen peroxide resulted in a product having a single resonance 0.37 ppm downfield from ETU and absorptions at 3210, and at 3160 and 2620 cm^{-1} in the infrared, suggestive of a secondary amine and an amine salt. Also present were absorptions at 1620 cm^{-1} characteristic of an imine and at 1210, 1060, and 650 cm^{-1} characteristic of an alkyl sulfonate. Based on spectroscopic properties and elemental analysis, this product was assigned the structure 2-imidazolin-2-yl sulfonate V.

To further characterize this product it was treated with ethereal diazomethane. The resulting derivative displayed two sharp singlets, one at 0.24 ppm (equivalent to four protons) downfield and one at 0.40 ppm (equivalent to six protons) upfield from the methylene singlet of ETU. The infrared spectrum of this derivative retained prominent absorptions at 1210, 1055, and 650 cm^{-1} (alkyl sulfonate) and at 1605 cm^{-1} (imine); however, amino absorptions were absent. The mass spectrum of the derivative displayed a weak molecular ion at M^+ 178, and prominent ions at m/e 130 and 114 corresponding to less of SO and SO_2 from the molecule; at m/e 98 corresponding to the *N,N*-dimethyl-2 imidazoline radical and m/e 64 (base peak) and 48 corresponding to SO_2 and SO, respectively. Based on these results and on elemental analysis, this product was assigned the structure *N,N*-dimethyl-2-imidazolin-2-yl sulfonate (VI).

Efforts to oxidize ETU to the level of a sulfinate were somewhat less successful. Several treatments of ETU (as a suspension in an inert solvent) with from 1.5 to 2.0 equivalents of 30% hydrogen peroxide resulted in a mixture of products containing sulfonate V, reactant, and an unknown. When the crude product mixture was dissolved in aqueous ammonia it tested positive to ammoniacal Naphthol Yellow S, a specific reagent for dithionite (Jelly, 1930). Control tests containing ETU or sulfonate V or a mixture of both were negative. This result was corroborated by electron paramagnetic resonance studies on the product mixture. When dissolved in 3 M ammonium hydroxide, at room temperature, the product mixture gave a paramagnetic signal at $g = 2.006$, strongly indicating the presence of SO_2^- radical ions in solution. The same signal was elicited when sodium dithionite was dissolved in the same solvent; however, neither ETU nor sulfonate V could be induced to decompose to a paramagnetic product. A paramagnetic signal at $g = 2.006$ from aminoiminomethane sulfinic acid has been used as evidence for the radical dissociation of this compound and eventual formation of urea (McGill and Lindstrom, 1977). These results favor the presence of 2-imidazolin-2-yl sulfinate (IX) in the oxidation product mixture. Further, it is difficult to see how one of the intermediates of scheme A (Figure 2) could decompose in base to an SO_2^- radical ion.

Isolation of the product having R_f 0.17 (Table I) by preparative TLC using methanol–acetic acid 9:1 led to a partially purified material whose spectrum showed a sharp singlet at 0.23 ppm downfield from ETU and absorption maxima at 269 and 318 nm in the ultraviolet when dissolved in 3 M ammonia. Sodium dithionite also displays

an absorbance maximum at 318 nm in this solvent. This product tested positive with Naphthol Yellow S, with ninhydrin and with Dragendorf reagent and afforded a pale blue coloration with Grote's reagent. These results are consistent with the postulated structure IX for this product.

That the mechanism of hypochlorite oxidation of ETU is similar to peroxide oxidation is demonstrated by the following series of experiments. When sulfonate V was treated with excess hypochlorite this substrate was found to rapidly consume 1 equivalent of oxidant (Table III) resulting in the formation of 1 equiv of ethyleneurea. This oxidation product was isolated by destroying excess hypochlorite (from a parallel run) with sodium sulfite, evaporating the crude reaction mixture to dryness and chromatographing the residues on a mixed bed ion-exchange column. Similar treatment of the dimethyl derivative VI resulted in a product which was identified from spectroscopic studies as *N,N*-dimethylethyleneurea (X), confirming N-methylation when sulfonate V was treated with diazomethane. These same oxidative decompositions of V and VI to EU to X, respectively, could also be effected in strong base by adding cadmium sulfate.

The oxidation of ETU by hypochlorite was also monitored by NMR. Calcium hypochlorite was added to ETU in D_2O containing excess sodium carbonate. The reaction mixture was centrifuged to remove precipitated calcium carbonate and the supernatant was monitored spectroscopically. Although addition of less than 4 equivalents of oxidant led to mixtures of products (product ratios were not stable with time) the reaction could be qualitatively monitored by this technique and demonstrated the appearance of two singlets at 0.23 and 0.38 ppm downfield and one singlet at 0.17 ppm upfield from ETU. Addition of standard sulfonate led to an increase in the downfield signal while addition of EU led only to an increase in the upfield signal. The crude product mixture also tested positive to Naphthol Yellow S, indicating the presence of sodium dithionite and suggesting the presence of sulfinate IX in the product mixture. In none of the runs was any evidence for the formation of Jaffe's base III observed, although this product is stable toward base hydrolysis (pH 11) for several hours and reacts only slowly with hypochlorite.

In summary these results indicate that oxidation in basic medium of ETU by hypochlorite and by hydrogen peroxide proceed via the same intermediates and may be represented by the following equations:

$$\text{ETU} + 4\text{NaOCl} + \text{Na}_2\text{CO}_3 \rightarrow \text{EU} + 4\text{NaCl} + \text{Na}_2\text{SO}_4 + \text{CO}_2$$

$$\text{ETU} + 3\text{H}_2\text{O}_2 \rightarrow \text{ETU}\cdot\text{O}_3 + 3\text{HOH}$$

The use of an alkaline medium to effect detoxification of ETU is preferable to neutral conditions in that the formation of Jaffe's base is avoided. Little is known about the biological activity of this oxidation product. It is possible, despite the stability observed above, that Jaffe's base could decompose to ETU on heating (e.g., industrial processing). Analogy for this decomposition is found in the work of Lechner and Gubernator (1953) who observed equal amounts of 1,1,3-trimethyl-2-thiourea and 1,1,3-trimethyl-2-urea from the hydrolysis of 1-(trimethylguanyl)-1,1,3-trimethyl-2-thiourea. Ethyleneurea is not tumorigenic (Sander, 1971) and together with sulfate represent innocuous oxidation end products.

Thus, oxidation using basic hypochlorite prior to industrial processing of vegetables which have been treated with EBDC fungicides would seem to be an effective means of removing contaminating surface residues of ETU. The application of such a technique could take the form of a prewash. The practicability of such a treatment will also depend on the efficacy with which the EBDC residues themselves are inactivated (to subsequent decomposition to ETU during processing). Preliminary investigations of the effect of hypochlorite on EBDC are promising and will be the subject of a future publication.

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