Nitrite Ion as a Nitrosating Reagent. Nitrosation of Morpholine and Diethylamine in the Presence of Formaldehyde

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The kinetics of the nitrosation of morpholine and diethylamine in the presence of formaldehyde has been studied at pH values between 6.5—8.2 and 6.9—8.7, respectively. The results are interpreted through a mechanism that implies the reaction between both the nitrite NO_2^- and iminium $R_2\overset{+}{N}{=}CH_2$ ions. The latter ion results from the dehydration of the conjugated acid of the carbinolamine, $R_2\overset{+}{N}HCH_2OH$, the initial product of the amine–formaldehyde reaction. The results allow the calculation of the equilibrium constants of formation of carbinolamine, R_2NCH_2OH, and methanediamine, $R_2NCH_2NR_2$ (only for the morpholine–formaldehyde system), and their conjugate acids.

It is well known that the formation of *N*-nitrosamines (which are powerful carcinogenic compounds) from the corresponding amine and nitrous acid in aqueous solution is best carried out at pH < 5.[1] These conditions are required to form nitrosating agents NOX (X = NO_2, Cl, Br, *etc.*) since neither nitrous acid itself, nor nitrite ion, interacts directly with secondary amines. The catalytic action of some aldehydes therefore has particular interest since it enables the nitrosation of secondary amines to take place under neutral and alkaline conditions.[2–5]

We have recently studied catalysis by FOR† of the nitrosation of DMA under both acidic and basic conditions.[6] The results suggest that the reaction occurs between nitrite ion itself and the *NN*-dimethylmethyleneammonium ion $Me_2\overset{+}{N}{=}CH_2$.[2] This ion results from the dehydration of protonated carbinolamine, $Me_2\overset{+}{N}HCH_2OH$, the initial product of the DMA–FOR reaction.

Roller and Keefer[2] have reported that in the presence of FOR, yields of nitrosamine varied according to PYR $\simeq$ PIP $\simeq$ DMA > DEA $\simeq$ DnPROP $\gg$ DiPROP, and were related to the steric accessibility of the nitrogen atom towards electrophilic attack. To check this hypothesis we have studied the nitrosation of DEA and MOR in the presence of FOR. These amines were chosen because values for the various equilibria between the amine and FOR are known[7–9] and our results could therefore be compared with these data.

Experimental

The inorganic chemicals (Merck, *pro analisi* grade) were dried and used without further purification. The formaldehyde (Merck, *pro analisi* grade) contained 10% of methanol as a stabilizer. MOR and DEA (Merck, 'for synthesis' grade) were added as their hydrochloride salts, after purification by recrystallization from methanol.

The kinetic measurements were carried out in a Kontron, model Uvikon-820, spectrophotometer with a thermostatically controlled cell holder. The temperature (25.0 ± 0.1 °C) was kept constant with a Heto O5E623 thermostat. Acidity was measured using a Radiometer, model PHM-82, pH meter with a GK2401B combined electrode.

The appearance of *N*-nitrosamines (NA) was followed spectrophotometrically at λ 260 nm where the molar absorptivities of the *N*-nitrosoamines, ε_{NA}, (1 074 ± 2 and 3 035 ± 6 $dm^3\ mol^{-1}\ cm^{-1}$ for NDEA and NMOR, respectively) are much greater than that of the nitrite ion (ε_{nit} 6 $dm^3\ mol^{-1}\ cm^{-1}$). Since $A_t - A_0 = (\varepsilon_{NA} - \varepsilon_{nit})[NA]$ where A is the absorbance and the subscripts t and 0 indicate time t and zero time, the initial reaction rate is given by $r_0 = (d[NA]/dt)_0 = (dA/dt)_0(\varepsilon_{NA} - \varepsilon_{nit})^{-1}$. The agreement between the expected value for A_0 (due to nitrite) and the experimental one ensures that r_0 is the real initial reaction rate. Under the experimental conditions used, an increment of 0.2 in the absorbance implies that the percentage of the reaction never exceeded 1%. Thus the graph of A *versus* t is a straight line, with a slope of r_0. The r_0 values were reproducible to within ± 3%.

Non-linear optimizations of the kinetic results were carried out following Marquardt's method[10] in a UNIVAC-1100 computer. The program minimizes $\Sigma[(r_{0i} - r_{0c})/r_{0i}]^2$ where r_{0i} is the experimental initial rate and r_{0c} is the calculated initial rate [equation (6)].

Results and Mechanism

At constant MOR and FOR concentrations a linear dependence of the intitial rate of MOR nitrosation, r_0, on nitrite concentration was observed (Figure 1).

At constant concentrations of nitrite and FOR, the effect of reaction pH was examined over the range 6.5—8.2 for ten different concentrations of MOR between 0.0131 and 0.618M. Some of these results are shown in Figure 2, where for clarity only two representative sets of experimental data are plotted. It can be seen that increasing the pH decreases the reaction rate and the effect is stronger at high MOR concentrations.

According to the mechanism proposed for DMA[6] the formation of nitrosamine results from the reaction between the iminium and nitrite ions [equation (1)]. This mechanism is

$$R_2\overset{+}{N}{=}CH_2 + NO_2^- \xrightarrow{k} R_2NNO + HCHO \quad (1)$$

consistent with the experimental dependence of r_0 upon $[\text{nitrite}]_0$.

Furthermore, according to the above mechanism the

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† The following acronyms are used: DEA, diethylamine; DiPROP, di-isopropylamine; DMA, dimethylamine; DnPROP, di-n-propylamine; FOR, formaldehyde; iBTA, isobutyraldehyde; MOR, morpholine; NDEA, *N*-nitrosodiethylamine; NMOR, *N*-nitrosomorpholine; PIP, piperidine; and PYR, pyrrolidine.

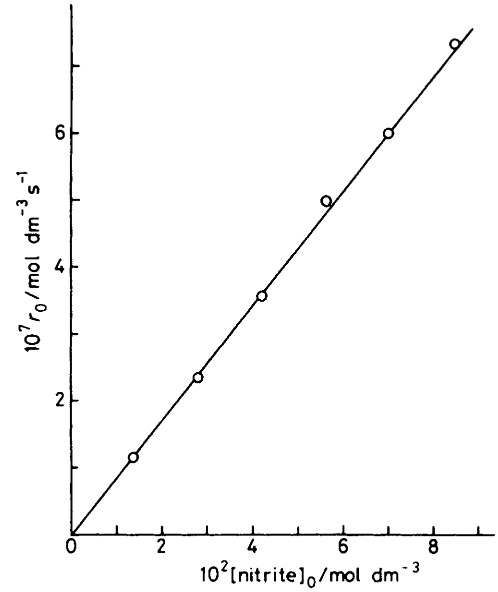

Figure 1. Dependence of initial rate of MOR nitrosation on nitrite concentration: $[\text{MOR}]_0 = 0.140\text{M}$, $[\text{FOR}]_0 = 0.126\text{M}$, pH = 6.95 ± 0.02, $t = 25\ °\text{C}$, $\mu = 0.5\text{M}$

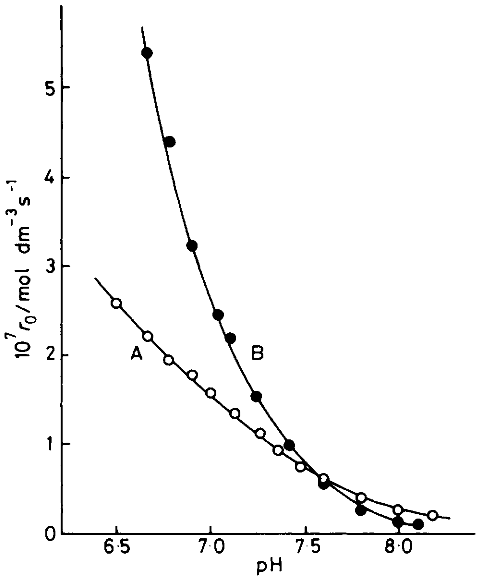

Figure 2. Influence of pH on the initial rate of MOR nitrosation at different amine concentrations: $[\text{MOR}]_0$(mol dm^{-3}) = 0.0654 (A) and 0.618 (B); $[\text{FOR}]^{10} = 0.635\text{M}$, $[\text{nitrite}]_0 = 0.0571\text{M}$, $t = 25\ °\text{C}$, $\mu = 1.0\text{M}$

concentration of iminium ion would be proportional to the concentration of protonated carbinolamine [equation (2)].

$$[\text{R}_2\overset{+}{\text{N}}\text{=CH}_2] = K_5[\text{R}_2\overset{+}{\text{N}}\text{H}_2\text{CH}_2\text{OH}] \qquad (2)$$

Thus the graph of r_0 *versus* $[\text{R}_2\overset{+}{\text{N}}\text{HCH}_2\text{OH}]_0$ should be a straight line, with its slope equal to $kK_5[\text{nitrite}]_0$.

By taking into account the published data about the MOR–FOR system,[7] [9] we can produce the Scheme, *i.e.*, the reaction products of the MOR–FOR interaction are the carbinolamine and the methanediamine, both of which are in equilibrium with their conjugated acids.

$$\begin{tikzcd}
\underset{\text{a}}{\text{R}_2\text{NH}} + \underset{\text{f}}{\text{H}_2\text{C(OH)}_2} \arrow[r, shift left, "K_1"] \arrow[d, shift right, "K_{a1}"'] & \underset{\text{m}}{\text{R}_2\text{NCH}_2\text{OH}} \arrow[l, shift left, "\text{H}_2\text{O}"] \arrow[r, shift left, "\text{R}_2\text{NH}\ K_3"] \arrow[d, shift right, "K_{a2}"'] & \underset{\text{d}}{\text{R}_2\text{NCH}_2\text{NR}_2} \arrow[l, shift left, "\text{H}_2\text{O}"] \arrow[d, shift right, "K_{a3}"'] \\
\underset{\text{a}^+}{\text{R}_2\text{NH}_2{}^+} + \text{H}_2\text{C(OH)}_2 \arrow[r, shift left, "K_2"] \arrow[u, shift right, "\text{H}^+"'] & \underset{\text{m}^+}{\text{R}_2\overset{+}{\text{N}}\text{HCH}_2\text{OH}} \arrow[l, shift left, "\text{H}_2\text{O}"] \arrow[r, shift left, "\text{R}_2\text{NH}\ K_4"] \arrow[u, shift right, "\text{H}^+"'] & \underset{\text{d}^+}{\text{R}_2\text{NCH}_2\overset{+}{\text{N}}\text{HR}_2} \arrow[l, shift left, "\text{H}_2\text{O}"] \arrow[u, shift right, "\text{H}^+"']
\end{tikzcd}$$

Scheme.

By taking account of the stoicheiometric conditions, $[\text{MOR}]_0 = x = [\text{a}] + [\text{a}^+] + [\text{m}] + [\text{m}^+] + 2[\text{d}] + 2[\text{d}^+]$ and $[\text{FOR}]_0 = y = [\text{f}] + [\text{m}] + [\text{m}^+] + [\text{d}] + [\text{d}^+]$, equation (3) can be readily deduced, where $A = (2y - x)/D + E[\text{H}^+]/F$;

$$[\text{a}^+]^3 + A[\text{a}^+]^2 + B[\text{a}^+] + C = 0 \qquad (3)$$

$B = E[\text{H}^+](y - x)/(DF) + [\text{H}^+]/(K_2F)$; $C = -[\text{H}^+]\cdot x/(K_2DF)$; $D = 1 + K_{a1}/[\text{H}^+]$; $E = 1 + K_{a2}/[\text{H}^+]$; and $F = K_3K_{a1}K_{a2}/[\text{H}^+] + K_{a1}K_4$.

Once equation (3) is resolved, $[\text{m}^+]$ is calculated from equation (4).

$$[\text{m}^+] = \frac{[\text{H}^+](x - D[\text{a}^+])}{E[\text{H}^+] + 2[\text{a}^+]F} \qquad (4)$$

It is evident that five independent constants are required to determine uniquely the system; the other two constants can be deduced from the relationships $K_{a1}/K_{a2} = K_2/K_1$ and $K_{a2}/K_{a3} = K_4/K_3$.

Values of 8.88, 6.09, and 7.38 were fixed for pK_{a1}, pK_{a2}, and pK_{a3}, respectively. These values, reported by Kallen *et al.*,[7] were determined at the same temperature (25 °C) and ionic strength (1.0M) used in this paper, and they correlate well with calculated values from Condon's[11] generalization of Hall's work.[12]

Values of kK_5, K_2, and K_4 were obtained by means of a non-linear optimization (see Experimental section) and are given in Table 1. The RMSD* of the 101 kinetic experiments carried out is 6.7%. Figure 3 shows that there is no systematic tendency of this deviation.

In order to study the influence of the ionic strength on the different constants, we have carried out kinetic runs at $\mu = 0.5\text{M}$, under conditions in which the concentrations of $\text{R}_2\text{NCH}_2\text{NR}_2$ and its conjugate acid were less than 4% of total MOR and FOR concentrations. Thus, if we suppose that [d] and $[\text{d}^+]$ are insignificant, $[\text{m}^+]$ can be calculated by means of equation (5),

$$[\text{m}^+] = \tfrac{1}{2}\{(x + y)/E + G/E^2 - \sqrt{[(x - y)/E]^2 + G^2/E^4 + 2G(x + y)/E^3}\} \qquad (5)$$

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* The RMSD is defined as $\{\Sigma[(r_{0\text{i}} - r_{0\text{c}})/r_{0\text{i}}]^2/(n - p)\}^{1/2}$ where n is the number of experimental points, and p is the number of optimization parameters.

Table 1. Equilibrium constants for adduct formation between formaldehyde and secondary amines at 25 °C

Compound	pK_{a1}	pK_{a2}	pK_{a3}	K_1/mol dm^{-3}	K_2/mol dm^{-3}	K_3/mol dm^{-3}	K_4/mol dm^{-3}	kK_5/mol^{-1} dm^3 s^{-1}	μ/mol dm^{-3}
MOR	8.56[a]	5.61		800	0.9	19.0			0.025—0.075
	8.85[b]	5.80—6.11		1 100 ± 200	1.9—2.1	24.4—30.3			1.0
				900		12.9—13.7			
	8.88[b]	6.09	7.38	925	1.51	18.9	362		1.0
	8.88[c]	6.09	7.38	1 273 ± 74	2.06 ± 0.12	18.9 ± 1.3	369 ± 27	(9.81 ± 0.23) × 10^{-4}	1.0
	8.70[c]	5.90		1 102 ± 31	1.747 ± 0.049			(1.45 ± 0.01) × 10^{-3}	0.5
DEA	11.02[a]			40					0.025—0.075
	11.02[c]	8.89 ± 0.12		48 ± 15	0.350 ± 0.053			(1.71 ± 0.21) × 10^{-5}	1.0
DMA	10.44[d]	7.714 ± 0.050		421 ± 41	0.792 ± 0.066			(3.99 ± 0.18) × 10^{-4}	2.0
								~7 × 10^{-4}	1.0

[a] Ref. 8. [b] Ref. 9. [c] This paper. [d] Ref. 6, 37 °C; a complete set of data is given in ref. 6.

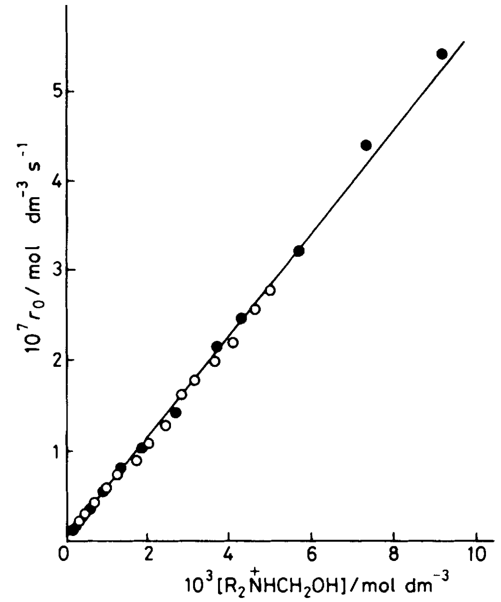

Figure 3. Dependence of initial rate of NMOR formation on protonated carbinolamine concentration (see text), under the experimental conditions described in Figure 2

where $G = D/K_2$. The conditions for these experiments were: $[\text{nitrite}]_0$ = 0.0142—0.0853M, $[\text{MOR}]_0$ = 0.0280—0.140M, $[\text{FOR}]_0$ = 0.0251—0.176M, and pH = 6.15—7.50. For a non-linear optimization to be applied a value of 8.70 was taken for pK_{a1} [13] and a value of 5.90 was fixed for pK_{a2}, *i.e.*, 2.80 units below pK_{a1} according to the correlations mentioned above and Kallen and Jencks' paper,[8] who have demonstrated that the difference (pK_{a1} − pK_{a2}) is independent of the ionic strength. Resulting values for kK_5 and K_2 are listed in Table 1. The RMSD of the 103 experimental points was 3.5%.

The nitrosation of DEA was investigated in three series of experiments. Firstly, for $[\text{DEA}]_0$ = 0.509M, $[\text{FOR}]_0$ = 0.503M, pH = 7.00, and μ = 1.0M, the initial concentration of nitrite was varied over the range 0.0143—0.0857M. The initial rate of formation of NDEA varied linearly with $[\text{nitrite}]_0$, the pseudo-first-order constant being (1.149 ± 0.009) × 10^{-6} s^{-1}. Secondly, for a fixed pH of 7.00 and $[\text{nitrite}]_0$ = 0.0714M, the concentrations of DEA and FOR were varied over the range 0.101—0.509M. Lastly, for $[\text{DEA}]_0$ = 0.509M, $[\text{nitrite}]_0$ = 0.0714M, and $[\text{FOR}]_0$ = 0.503M, the pH of the reaction medium was varied over the range 6.90—8.70. These results show that r_0 is almost independent of pH, decreasing by only 15%. The kinetic runs were carried out in phosphate buffers (0.0507M) which had no effect on the reaction rate.

A non-linear optimization was carried out in accordance with equation (5). Values of kK_5, K_2, and pK_{a2} are given in Table 1, where the high error (25% in K_{a2}) in the constants is due to a certain correlation between the constants resulting from the optimization process in spite of the low RMSD, which is 4.1% (experimental points 63). As Figure 4 shows, the dependence of r_0 on the concentration of $Et_2\overset{+}{N}HCH_2OH$ is linear.

Discussion

Following previous papers (reference 7 and references cited therein) the zwitterionic form of the carbinolamine $R_2\overset{+}{N}H$-CH_2O^-, the cationic dihydroxymethylamine $R_2\overset{+}{N}(CH_2OH)_2$, and the diprotonated methanediamine $R_2\overset{+}{N}HCH_2\overset{+}{N}HR_2$ [values of 1.0—1.3 and 2.2 are estimated for the pK_a corresponding to $R_2 = (CH_2)_2O(CH_2)_2$ and R = Me, respectively] were omitted from the Scheme. For the MOR–FOR system, Kallen *et al.* reported that equilibrium is attained in less than 5 min and we added the nitrite at least 40 min after the amine–FOR solutions were prepared at the required pH. Using the initial rate method (the reaction was never followed further than 1%) we can consider that the system remains unperturbed. This is supported by the values obtained for the equilibrium constants, which agree with those reported by other workers (Table 1). The value obtained for pK_{a2} corresponding to diethylaminomethanol is comparable with the value of 8.80 calculated from Hall's correlation,[12] with an allowance of 0.34 units for the ionic strength used in this work.

Furthermore, the published value[7] for K_1K_3 of 14.3, corresponding to DEA–FOR, justifies the use of equation (5) instead of equations (3) and (4) for this system.

The equilibrium constants for the formation of neutral and cationic carbinolamine, K_1 and K_2, for the three amines listed in Table 1 show that they are almost independent of the polar character of the amine but are influenced by the steric accessibility of the nitrogen atom. This aspect is well illustrated in the literature.[8,14,15] Table 2 shows these constants are also influenced by steric effects owing to the alkyl group of the aldehyde. Thus, it is easy to understand why 2,2-dimethylpropionaldehyde failed to promote DEA nitrosation under similar conditions to those for FOR [2] and the order of reactivity for several amines found by Roller and Keefer.

Our results agree quite well with those reported by Roller and Keefer. Using equations (5) and (6), the constants listed in

$$r_0 = kK_5[\text{nitrite}]_0[R_2\overset{+}{N}HCH_2OH]_0 \qquad (6)$$

Table 1 for DEA and the experimental conditions used by Roller and Keefer ([DEA] = 0.05M, [FOR] = 0.05M, [nit-

Table 2. Equilibrium constants for adduct formation between different aldehydes and secondary amines

	Aldehyde			
	MeCHO[a]		EtCHO[b]	iBTA[c]
Amine	$K_1{}^d$/mol dm^{-3}	$K_2{}^e$/mol dm^{-3}	K_1/mol dm^{-3}	K_1/mol dm^{-3}
Morpholine	20.5	0.056	13 ± 1	0.64
Piperazine				1.12
Dimethylamine				1.70 ± 0.05

[a] B. Gaux and P. Le Hénaff, *Bull. Soc. Chim. Fr.*, 1972, 2501. [b] C. Bordat, B. Gaux, and P. Le Hénaff, *C. R. Hebd. Seances Acad. Sci.*, 1975, **281**, 579. [c] J. Hine and J. Mulders, *J. Org. Chem.*, 1967, **32**, 2200. [d] $K_1 = [R_2NC(R')HOH]/[R_2NH][R'CHO]$. [e] $K_2 = [R_2\overset{+}{N}HC(R')HOH]/[R_2\overset{+}{N}H_2][R'CHO]$.

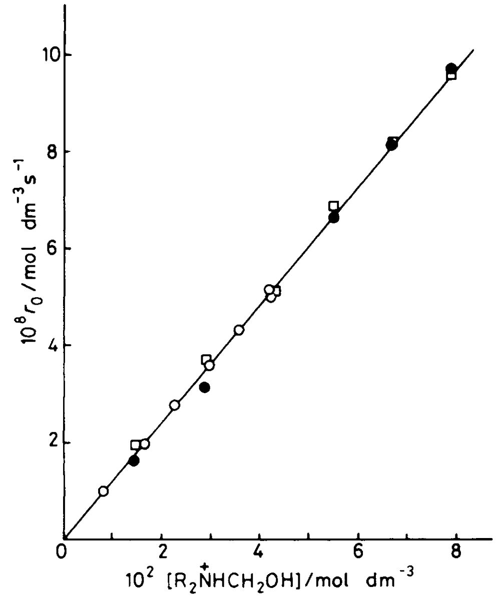

Figure 4. Dependence of r_0 on the concentration of $Et_2\overset{+}{N}HCH_2OH$ at pH = 7.00, [phosphate] = 0.0507M, $[\text{nitrite}]_0$ = 0.0714M, t = 25 °C, μ = 1.0M; □, $[\text{FOR}]_0$ = 0.503M, $[\text{DEA}]_0$ = 0.102—0.509M; ● $[\text{DEA}]_0$ = 0.509M; and ○ $[\text{DEA}]_0$ = 0.255M, $[\text{FOR}]_0$ = 0.101—0.503M

rite] = 0.2M, pH = 7.0 in 0.5M phosphate buffer at 24 °C) a value of 2.9×10^{-9} mol dm^{-3} s^{-1} for r_0 is calculated. Assuming r_0 remains constant for 17 h, which is not unreasonable in view of the small extent (0.35%) of reaction, the calculated yield of 1.8×10^{-4} moles of NDEA or 0.36% agrees well with the experimental one.

The results of Archer *et al.*[3] are in disagreement with those reported here. Working at [FOR] = 0.05M, [nitrite] = 0.2M, and pH = 6.5 at 25 °C, they found that the initial nitrosation of MOR is fastest for [MOR] = 0.05M. Higher concentrations of MOR (*e.g.*, 0.20M), decreased the initial rate by 34% with respect to the maximum value. We have not observed a similar inhibitory effect up to an amine concentration to 0.618M.

Although our results could be interpreted in terms of a direct reaction between nitrous acid and the neutral carbinolamine we have rejected this interpretation because nitrous acid itself does not act as a nitrosating agent towards primary and secondary amines.[1,16] There is no reasonable basis to suppose that carbinolamine (a tertiary amine) could directly react with nitrous acid.

Finally, we want to point out that we cannot decide whether the rate-limiting step is the self-decomposition of the α-amino nitrite ester R_2NCH_2ONO,[2] formed in a previous equilibrium from $R_2\overset{+}{N}{=}CH_2$ and NO_2^-, or the reaction between both ions.[6]

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