Degradation of Gabexate Mesilate by Sodium Bisulfite[1)]

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The hydrolysis of gabexate mesilate (GM) in aqueous solution was found to be accelerated by sodium bisulfite (SBS). The hydrolysis in the presence of SBS was pseudo-first-order and the action of SBS was found to be catalytic. In the pH range of 5.0—7.5, the dominant degradation process was considered to be a reaction between gabexate cation (G^+) and sulfite ion. The rate constant for the catalytic hydrolysis of G^+ with sulfite ion was 502.6 $M^{-1} h^{-1}$ at 25 °C and $\mu = 0.5$. The effects of temperature and concentration of SBS on the hydrolysis were evaluated. From these findings, we produced a nomograph to predict the stability of GM in the parenteral admixture containing SBS as an antioxidant.

Keywords sodium bisulfite; gabexate mesilate; degradation; parenteral admixture compatibility; stability estimation

Sodium bisulfite (SBS), used as a stabilizer in pharmaceutical preparations for injection, is known to degrade various drugs such as thiamine,[2)] epinephrine,[3)] catecholamines,[4)] adrenocortical hormones,[5)] morphine,[6)] fluorouracil,[7)] ascorbic acid,[8)] penicillins and cephalosporins.[9)] We have developed methods for determination of SBS and studied the degradation of fulsultiamine and thiamine by SBS.[10-12)]

Though SBS reacts with various drugs, there are no reports on its reactivity with ester drugs, except for acceleration of the hydrolysis of aspirin.[13)] In the present study, we chose, among ester drugs frequently used for injection, gabexate mesilate (GM), a protease inhibitor, and meclofenoxate hydrochloride (MH), a drug used to treat disturbance of consciousness. A solution of GM or MH alone is readily hydrolyzed[14,15)] (Chart 1), and the stability of both drugs in the presence of SBS was studied. The rate of hydrolysis of MH was not accelerated by SBS, but that of GM was accelerated by SBS concentration-dependently.

Kinetic analysis of the reaction of GM with SBS revealed that the reaction between gabexate cations (G^+) and sulfite anions (SO_3^{2-}) is the main reaction in the pH range of 5.0—7.5. Based on the obtained data, a nomograph was produced for evaluation of compatibility when GM is mixed with an infusion or parenteral admixture containing SBS. The mechanisms of acceleration of the hydrolysis of ester drugs by SBS and the causes of the differences in reactivity to SBS between GM and MH were also considered.

Experimental

Materials Gabexate mesilate and MH were standard products supplied by Ono Pharmaceutical Co., Ltd. And Dainippon Pharmaceutical Co., Ltd., respectively. Sodium bisulfite, 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), and the other agents were commercial products of special grade.

As buffer solutions, Michaelis buffer (0.05 M acetate, pH 5.0—5.5) and Sörensen buffer (0.05 M phosphate, pH 6.0—7.5) were adjusted with sodium chloride to an ionic strength of 0.5. Distilled water that had been boiled and saturated with nitrogen was used.

Kinetic Procedures The stability of GM solution (1×10^{-3} M) buffered to pH 5.0—7.5 in the presence and absence of SBS (1×10^{-3}, 2×10^{-3} M) was studied by the isothermal method and temperature-raising method under a nitrogen atmosphere. In the temperature-raising method, the temperature was raised linearly from 5 to 40 °C at a rate of 0.1 °C/h by using a BCP-100 type thermostatic water bath (Yamato Scientific Co., Ltd.). A conventional thermostatic water bath was used in the isothermal method.

Determination of GM Gabexate mesilate was measured by high-performance liquid chromatography (HPLC) according to the method of Kobo[16)] with some modification.

1) Equipment: A Hitachi 655A-11 high-performance liquid chromatograph, a Hitachi 655A ultraviolet (UV) detector, and a Shimadzu C-R3A chromatopack were used.

2) Measurement Conditions: column, YMC-pack AM311ODS (4.6 mm i.d. × 100 mm); mobile phase, methanol–water–5% (w/v) sodium lauryl sulfate–5% (w/v) sodium 1-heptanesulfonate–acetic acid (540 : 220 : 4 : 2 : 1); flow rate, 0.7 ml/min; column temperature, 250 °C; detection wavelength, 258 nm; and sensitivity, 0.10 AUFS. As an internal standard, *n*-butyl *p*-hydroxybenzoate was used, and a 10 μl aliquot was injected into HPLC.

Determination of MH Meclofenoxate hydrochloride was measured using equipment similar to that used in the case of GM.

Measurement conditions: column, YMC A-312 ODS 5 μ (6 mm i.d. × 150 mm) (Yamamura Chem. Co., Ltd.); mobile phase, 0.01 M acetate buffer (pH 3.0)–acetonitrile (65 : 35); flow rate, 2.0 ml/min; column temperature, 25 °C; detection wavelength, 280 nm; and sensitivity, 0.16 AUFS. As an internal standard, n-propylbenzoate was used, and a 20 μl aliquot was injected into the HPLC.

Results and Discussion

Determination of GM by HPLC Methods for the determination of GM by HPLC have been reported by Nishijima *et al.*[17)] and Kobo.[16)] We compared these two methods and found the method of Kobo to be superior. However, this method has not been adequately evaluated as a quantification method, and since the column temperature

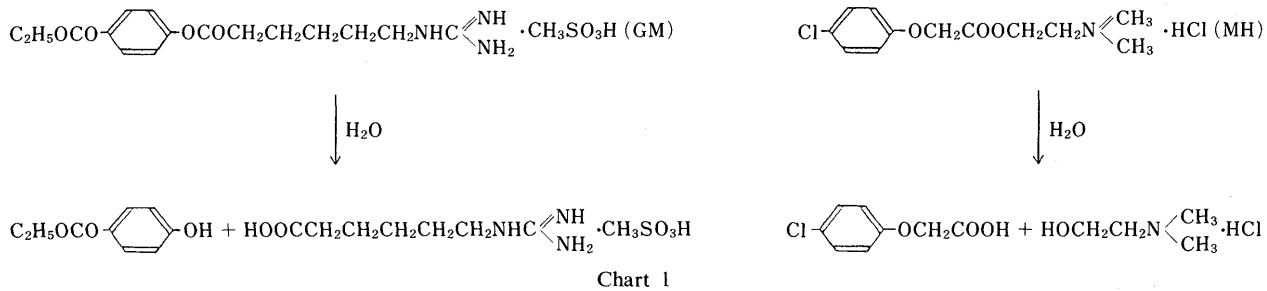

Chart 1

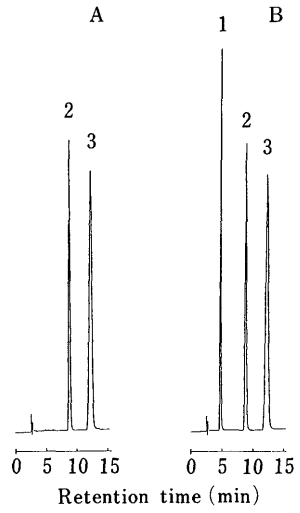

Fig. 1. HPLC of GM

A) GM standard; B) GM in sample solution. Peak 1, ethyl *p*-hydroxybenzoate; peak 2, internal standard (*n*-butyl *p*-hydroxybenzoate); peak 3, GM.

applied is 45 °C, some degradation of GM might occur in the column. Therefore, conditions for measurement at a lower temperature were studied. The conditions described in Experimental allowed good separation at a column temperature of 25 °C (Fig. 1). Guanidinocaproic acid, a degradation product of GM, showed no peak because it has no absorption in the UV region. A calibration curve was produced using 25—500 μg/ml solutions of GM based on the peak area obtained by HPLC. The coefficients of variation for GM at the concentrations of 25 and 400 μg/ml were 0.7% ($n=6$) and 0.5% ($n=6$), respectively.

Stability of GM in the Presence and Absence of SBS The stability of GM in the presence and absence of SBS was studied by the isothermal method and the temperature-raising method. In the pH range of 6.0—7.5 the degradation rate of GM was measured by the isothermal method. Since GM was rather stable at pH 5.0 and 5.5, the degradation rate of GM in this pH range was measured by the temperature-raising method. HPLC of GM under various conditions in the presence of SBS revealed a peak due to ethyl *p*-hydroxybenzoate as a degradation product. Gabexate mesilate is known to be hydrolyzed into ethyl *p*-hydroxybenzoate and guanidinocaproic acid in the absence of SBS.[14] In the presence of SBS, the same degradation products seemed to be formed.

a) Isothermal Method The degradation rate constant of GM was measured in the presence (k_{obs}) and absence (k_o) of SBS by the isothermal method at pH 6.0—7.5 and at 5, 25, and 40 °C. The results show that hydrolysis of GM in both the presence and absence of SBS is a pseudo-first-order reaction. Figure 2 shows the results at pH 6.0 and at 25 °C. Measurements of SBS in the samples by the DTNB method[18] revealed no consumption in the reaction, demonstrating catalytic action of SBS. Rate constants of GM obtained at various temperatures in the presence or absence of SBS at a constant pH of the sample were plotted according to the Arrhenius equation. The results at a pH of 6.0 (Fig. 3) show good linearity both in the presence and absence of SBS. The activation energy (E_a) was obtained from the slope of the line.

b) Temperature-Raising Method The degradation rate

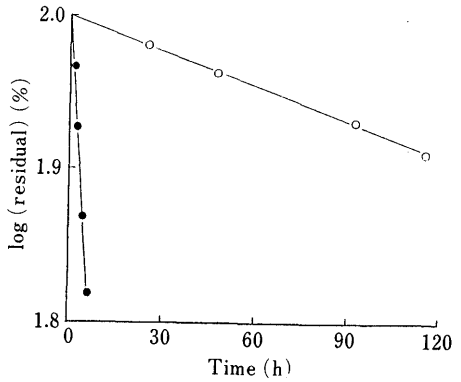

Fig. 2. First-Order Plots for the Degradation of GM in the Presence and Absence of SBS in 0.05 M Phosphate Buffer (pH 6.0) at 25°C and $\mu=0.5$

Initial concentration of GM, 1.0×10^{-3} M; ○, $[SBS]_{total}=0$ M; ●, $[SBS]_{total}=2.0\times10^{-3}$ M.

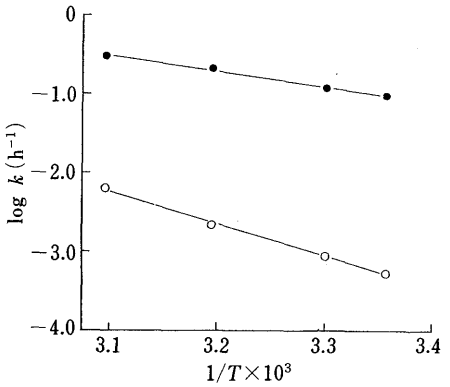

Fig. 3. Arrhenius-Type Relationship between the Degradation Rate Constant of GM and Temperature in the Presence and Absence of SBS in 0.05 M Acetate Buffer (pH 6.0) and $\mu=0.5$

Initial concentration of GM: 1.0×10^{-3} M; ○, $[SBS]_{total}=0$ M; ●, $[SBS]_{total}=2.0\times10^{-3}$ M.

TABLE I. Activation Energies (E_a) for the Hydrolysis of GM in the Presence and Absence of SBS

pH	E_a (kcal mol^{-1})	
	GM	GM–SBS
5.0	21.38	6.92
5.5	22.45	7.65
6.0	18.77	8.95
6.5	17.74	9.03
7.0	21.80	8.75
7.5	23.80	10.39
Average	20.99±2.09	8.62±1.10

constant of GM (k_o or k_{obs}) and the activation energy (E_a) in the presence or absence of SBS were calculated in the samples at pHs 5.0 and 5.5 by the linear temperature-raising method. Ohkusa and Kinuno[19] presented Eq. 1 as the relationship between the temperature and time and analyzed the constituent concentration using Eq. 2, where $a=1/(2.303\cdot T_o)$, T_o is the absolute temperature from which the increase of temperature is initiated, T_t is the absolute temperature after t hours, b is a constant determined by a, K_I is the rate constant when the increase of temperature is initiated, E_a is activation energy, $\boldsymbol{R}$ is the gas constant, and f is constituent concentration. The results obtained by the

isothermal method demonstrated that hydrolysis of GM both in the presence and absence of SBS is a pseudo-first-order reaction. Therefore, the constituent concentration was expressed at 2.303 log (C_o/C_t),[19] where C_o is the initial concentration of GM and C_t is that after t hours.

$$\frac{1}{T_o}-\frac{1}{T_t}=2.303\cdot a\cdot\log(1+b\cdot t) \quad (1)$$

$$\log f=\log k_t-\log b\cdot\left(1+\frac{E_a}{R}\right)+\left(1+\frac{E_a}{R}\right)\cdot\log(1+b\cdot t) \quad (2)$$

Using Eq. 2, log f, *i.e.*, log(2.303 log C_o/C_t) was plotted against $\log(1+b\cdot t)$ and a line with a slope of $(1+E_a/R)$ was obtained (Fig. 4). The activation energy (E_a) was calculated from the slope. The rate constant (k_t) at the initiation of the temperature increase was calculated from the point of intersection obtained by extrapolating log $(1+b\cdot t)$ to 0.

The activation energies of GM in the presence and absence of SBS at pH 5.5—7.5 obtained by the isothermal and temperature-raising methods are shown in Table I. In this relatively narrow range of pH, the E_a values in the absence and presence of SBS are 20.99 ± 2.09 and 8.62 ± 1.10 kcal mol^{-1}, respectively. The E_a in the presence of SBS was about 2/5 of that in the absence of SBS, demonstrating catalytic action of SBS.

Effects of SBS Concentration Stability was evaluated in solutions containing GM at a constant concentration and SBS at various concentrations at 25 °C and pH 6.0. The degradation rate constant of GM (k_{obs}) was plotted against the total SBS concentration ($[SBS]_{total}$) as shown in Fig. 5. The degradation rate of GM was proportional to the total concentration of SBS. The rate constant for the catalytic hydrolysis of GM by SBS (k_{SBS}) obtained from the slope of the line was 39.9 M^{-1} h^{-1}. The point of intersection between this line and the y axis ($[SBS]_{total}=0$) was 5.37×10^{-4} h^{-1}, which corresponds to the rate constant of GM alone (k_o). Therefore, the rate constant of GM in the presence of SBS (k_{obs}) is represented as Eq. 3.

$$k_{obs}=k_o+k_{SBS}\cdot[SBS]_{total} \quad (3)$$

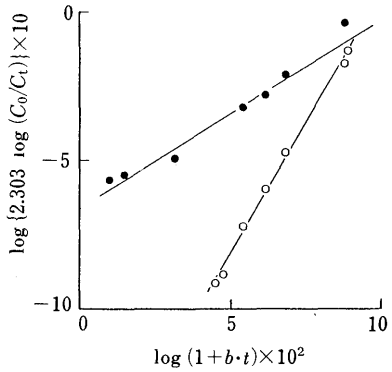

Fig. 4. Integral Analysis of the Temperature-Raising Method for the Degradation of GM in the Presence and Absence of SBS in 0.05 M Acetate Buffer (pH 5.5) and $\mu=0.5$

Rate of temperature increase, 0.1°C/h; initial concentration of GM: 1.0×10^{-3} M; ○, $[SBS]_{total}=0$ M; ●, $[SBS]_{total}=2.0\times10^{-3}$ M.

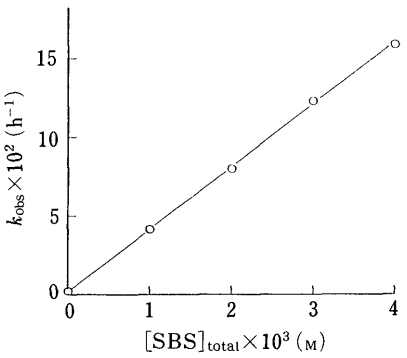

Fig. 5. Relationship between the Total Concentration of SBS and the Apparent First-Order Rate Constant (k_{obs}) for the Degradation of GM by SBS in 0.05 M Phosphate Buffer (pH 6.0) and at 25 °C

Initial concentration of GM, 1.0×10^{-3} M.

Effect of pH The effects of pH on the stability of GM (1×10^{-3} M) in the presence and absence of SBS (1×10^{-3} and 2×10^{-3} M) were studied at various temperatures. The pH-profiles of GM at a concentration of 1×10^{-3} M at 25 °C in the presence (2×10^{-3} M) and absence of SBS are shown in Fig. 6, where the rate constants are expressed as logarithmic values. Curve 4 represents the rate constant of GM alone (k_o), and curve 2 represents that of GM in the presence of SBS (k_{obs}). Curve 3 shows the rate constant ($k_{obs}-k_o$) dependent on SBS alone at a concentration of 2×10^{-3} M.

Since the pK_a for the guanidino radical of arginine is 12.48,[20] the pK_a for the same radical of GM was expected to be similar. Thus, GM was considered to be present in the form of gabexate cations (G^+) in the pH range (5.0—7.5) employed in this experiment. Therefore, the concentration of G^+ was considered to be equal to the total GM concentration. Similarly, based on the dissociation constant of SBS ($K_1=1.72\times10^{-2}$ and $K_2=6.24\times10^{-8}$),[21] most of the SBS in this pH range was considered to be present as bisulfite ions (HSO_3^-) or sulfite ions (SO_3^{2-}). Pyrosulfite ions produced from bisulfite ions were excluded from the analysis because their amount is small.[8]

From the above results, the hydrolysis of GM by SBS is assumed to proceed as shown in Eq. 4. Then, Eq. 5 can be derived from Eqs. 3 and 4. The ionic species of SBS are represented by Eqs. 6—8.[21] From Eqs. 6—8, Eq. 5 may be converted to Eq. 9.

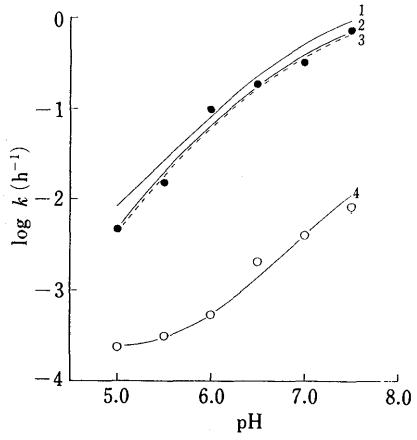

Fig. 6. pH-Profiles of the Degradation of GM in the Presence and Absence of SBS at 25°C and $\mu=0.5$

Initial concentration of GM, 1.0×10^{-3} M; ○, $[SBS]_{total}=0$ M; ●, $[SBS]_{total}=2.0\times10^{-3}$ M. 1, theoretical curve for SBS-dependent degradation of GM in GM–SBS solution; 2, curve for degradation of GM in GM–SBS solution (k_{obs}); 3, curve for SBS-dependent degradation of GM in GM–SBS solution ($k_{obs}-k_0$); 4, curve for degradation of GM in solution (k_0).

$$G^+ \underset{k_{SO_3^{2-}}\ SO_3^{2-}}{\overset{k_{HSO_3^-}\ HSO_3^-}{\rightleftharpoons}} \text{products} \quad (4)$$

$$k_{obs}-k_0=k_{HSO_3^-}\cdot[HSO_3^-]+k_{SO_3^{2-}}\cdot[SO_3^{2-}] \quad (5)$$

$$[SBS]_{total}=[H_2SO_3]+[HSO_3^-]+[SO_3^{2-}] \quad (6)$$

$$K_1=\frac{[H^+]\cdot[HSO_3^-]}{[H_2SO_3]} \quad (7)$$

$$K_2=\frac{[H^+]\cdot[SO_3^{2-}]}{[HSO_3^-]} \quad (8)$$

$$k_{obs}-k_0=\left(\frac{k_{HSO_3^-}\cdot K_1\cdot[H^+]+k_{SO_3^{2-}}\cdot K_1\cdot K_2}{[H^+]^2+K_1\cdot[H^+]+K_1\cdot K_2}\right)[SBS]_{total} \quad (9)$$

Where $k_{HSO_3^-}$ is the catalytic rate constant in the hydrolysis of G^+ by bisulfite ions, and $k_{SO_3^{2-}}$ is that by sulfite ions. Equation 9 was solved by the nonlinear least-squares approach using the simplex method,[22] and $k_{HSO_3^-}=1.7\,M^{-1}h^{-1}$ and $k_{SO_3^{2-}}=502.6\,M^{-1}h^{-1}$ were obtained. These results show that the accelerating effect of sulfite ions on the hydrolysis of GM was 300 times greater than that of bisulfite ions.

Prediction of Stability of GM In an admixture for injection in a relatively small range of pH, as mentioned above, changes in pH produce only slight changes in activation energy for the hydrolysis of GM. In addition, there was a linear relationship between the degradation rate and concentration of sulfite ions. Therefore, based on the pH-profiles, a nomograph for estimating the utilizable time of an admixture for injection containing GM was produced (Fig. 7). The time required for reduction of the remaining ratio of the drug to 90% ($t_{0.9}$) was defined as the utilizable time. Using this nomograph, the stability of GM in parenteral admixtures containing SBS at various concentrations could be estimated readily from the pH and temperature. This nomograph is useful for evaluating the compatibility of parenteral admixtures containing SBS or for chooseing optimum conditions of storage. Assuming that generally 500 ml of parenteral admixture containing GM takes 4 h for mixing, transportation and administration in the hospital, use of an admixture containing GM with a $t_{0.9}$ of less than 4 h is not appropriate. In Fig. 7, when the GM concentration is 200 mg/500 ml (*ca.* 1×10^{-3} M), storage for 13 h is possible at a room temperature of 25 °C and even a pH of 7.5 in the absence of SBS (curve 2). However, when GM is mixed with infusion containing SBS at a concentration of 2×10^{-3} M, infusion at pH of 5.5 or more at this temperature is considered to be inappropriate because the $t_{0.9}$ is less than 4 h (curves 6 and 7).

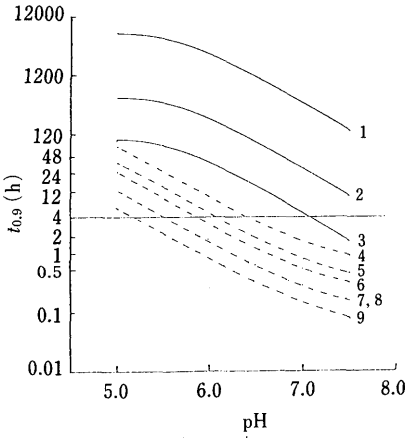

Fig. 7. Nomograph for Estimation of Utilizable Time ($t_{0.9}$) of GM in Admixtures Containing Various Concentration of SBS at Various Temperatures and pHs

Initial concentration of GM was 1.0×10^{-3} M. 1, GM solution at 5°C; 2, GM solution at 25°C; 3, GM solution at 40°C; 4, GM with 1.0×10^{-3} M SBS at 5°C; 5, GM with 2.0×10^{-3} M SBS at 5°C; 6, GM with 1.0×10^{-3} M SBS at 25°C; 7, GM with 2.0×10^{-3} M SBS at 25°C; 8, GM with 1.0×10^{-3} M SBS at 40°C; 9, GM with 2.0×10^{-3} M SBS at 40°C.

Stability of MH in the Presence and Absence of SBS The stability of MH, another ester drug, was also evaluated. The stability of MH of 2×10^{-3} M in the presence and absence of SBS of 2×10^{-3} M at 30 °C was measured by the isothermal method at pHs of 3.9 (0.05 M acetate buffer) and 5.8 (0.05 M phosphate buffer). In contrast to GM, the rate of degradation of MH was not affected by the presence or absence of SBS. The pseudo-first-order degradation rate constant in the presence of SBS was similar to that in the absence of SBS; it was $6.6\times10^{-3}\,h^{-1}$ at pH 3.9 and $1.6\times10^{-1}\,h^{-1}$ at pH 5.8. Since the pK_a for MH is 8.3,[15] NH was considered to be present as meclofenoxate cations (M^+), as with GM at the examined pHs.

Difference in Reactivity between GM and MH The hydrolysis of GM was accelerated in the presence of SBS, but that of MH was not. This may be caused by the structural difference that GM is a phenylester derivative similar to aspirin, while MH is an alkylester derivative. Assuming that the hydrolysis of GM is a general nucleophilic addition elimination reaction, G^+ seems to be attacked more strongly by sulfite ion than by hydroxy ion,[23] resulting in formation of a sulfite-added intermediate.[23] Since a substituted phenoxide radical is readily eliminated,[24] hydrolysis seems to be accelerated. However, since it is considered that the alkoxide radical of MH is hardly eliminated, and this elimination is the rate-limiting step, changes in the nucleophile type may not affect the degradation rate.

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