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Taurine–nitrite interaction as a precursor of alkylation mechanisms

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ABSTRACT

Taurine (2-aminoethanesulphonic acid) is an amino acid-like-compound widely used as an ingredient in some nutraceuticals and energy drinks. Here the interaction of taurine (Tau) with nitrite was investigated. The reactions were carried out mimicking the conditions of the stomach lumen. The conclusions drawn are as follows: (i) Nitrite showed nitrosating capacity on Tau. The rate equation was $v_N = k_{obs}[Tau]_o[nitrite]_o^2$, this result suggesting that the yield of nitrosation products in the human stomach would increase sharply with higher nitrate/nitrite intakes; (ii) the experimental results suggest a mechanism for the nitrosation, whose rate-limiting step is bimolecular attack by N_2O_3; (iii) the nitrosation of taurine affords ethanesultone (ES), which displays alkylating capacity on the nucleophile 4-(*p*-nitrobenzyl)pyridine (NBP), a trap for alkylating agents with nucleophilic characteristics similar to those of DNA bases. Although the NBP alkylation rate for ethanesultone is much higher than those for carcinogenic four-membered ring lactones, resulting in the nitrosation of amino carboxylic acids, the fraction of ES-forming adduct with NBP is much smaller; (iv) in spite of the low risk to human health, since the stomach lumen conditions could be a favourable medium for Tau nitrosation, attention should be paid to potential situations of the concurrence of high contents of taurine and nitrite/nitrate in the diet.

1. Introduction

Taurine (Tau), or 2-aminoethanesulphonic acid (CAS 91105-79-2), is a semi-essential amino acid-like compound present in human tissues and the bloodstream, where it is involved in biological mechanisms such as chemoprotection (Gurer, Ozgunes, Saygin, & Ercal, 2001), neurotransmission (Olive, 2002), and calcium homeostasis (Palmi et al., 1999). Taurine is ubiquitous in nature, but its distribution and concentration differ among biological organisms. Its role as an antioxidant and osmoregulator has been recognised (Huxtable, 1992). The anti-inflammatory (Ward et al., 2006) and anti-anxiety (Zhang & Kim, 2007) actions of this compound have also been reported.

Taurine is present in the diet (especially in meat and seafood), and it is widely used as an ingredient in some nutraceuticals and energy drinks.

Despite the large body of evidence for the beneficial effects of taurine supplementation in a variety of diseases, the underlying modifying action of taurine with respect to either molecular or biochemical mechanisms is almost totally unknown (Della Corte et al., 2002).

Since taurine has a primary amine group, it can be expected to react with nitrite, undergoing nitrosation under stomach lumen conditions (García-Santos, González-Mancebo, Hernández-Benito, Calle, & Casado, 2002).

N-Nitroso compounds are unique among carcinogenic agents in that they are active in all species and because there is a broad spectrum of target cells and organs in which they are able to induce cancer. The formation of nitroso compounds can occur in food (when preserved with nitrite in order to inhibit the growth and toxin production of *Clostridium botulinum* in meat); in the environment, and in the digestive tract (especially in the stomach). Some *N*-nitroso compounds are even synthesised by plants, although most of these are formed incidentally through the nitrosation of amines (Lijinsky, 1992; Loeppky & Michejda, 1994; Mirvish, 1975).

Biologists have mainly been interested in the pathogenic mechanisms in which nitroso compounds are involved (Walters, 1973), whereas chemists have been more interested in their mechanisms of formation (González-Jiménez, Arenas-Valgañón, Calle, & Casado, 2011; Williams, 2004), and hence in ways to block or inhibit them (González-Mancebo, García-Santos, Hernández-Benito, Calle, & Casado, 1999; Loeppky & Michejda, 1994).

Not all nitroso compounds formed in nitrosation reactions are stable and they may sometimes evolve into compounds that are

Abbreviations: Tau, Taurine; BBL, β-butyrolactone; BPL, β-propiolactone; NBP, 4-(*p*-nitrobenzyl)pyridine; ES, ethanesultone; ITA, isethionic acid; DIK, diketene.

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potential alkylating agents. This is the case of the nitroso compounds formed in the nitrosation of certain amino carboxylic acids with the –NH_2 group, which evolve into lactones (García-Santos, Calle, & Casado, 2001) (Fig. 1). β-Butyrolactone (BBL) and β-propiolactone (BPL), formed in the nitrosation of β-aminobutyric acid and β-alanine, respectively, are classified as "possibly carcinogenic to humans" (group 2B) by the IARC (IARC, 1999). Accordingly, mechanistic studies of the nitrosation reactions of compounds analogous to amino acids are of interest.

Since, (i) to our knowledge no studies have addressed the influence of the substitution of the carboxyl group of amino acids by the sulphonic group on their reactivity in nitrosation mechanisms; (ii) the nucleophilicities of COOH and SO_3H groups, as well as the electronic inductive effect of sulfonates and carboxylates, are different (sulphonic acids are about one million times-six pK_a units-more acidic than carboxylic acids), here we were prompted to investigate these issues.

2. Materials and methods

2.1. General

A Shimadzu UV-2401PC spectrophotometer (Japan) with a thermoelectric six-cell holder temperature system was used. The reaction temperature was kept constant (±0.05 °C) with a Lauda Ecoline RE120 thermostat (Germany). A Metrohm pH lab 827 pH-meter (Switzerland) was used. Electrospray ionisation mass spectra were recorded on a Waters ZQ4000 spectrometer (USA). Numerical treatment of the data was performed using Sigma Plot 9.0 software.

^{1}H NMR spectra were recorded with a Bruker Avance 400 (400 MHz) spectrometer (Germany) equipped with an indirect probe with Z-gradients. The compounds were dissolved in D_2O and the residual solvent peak (HDO) used as internal reference.

Taurine (>99%), NBP (98%), isethionic acid (98%) and Et_3N (99%) were obtained from Sigma–Aldrich (Germany); $NaNO_2$ and $NaClO_4$ were from Merck (Germany); $HClO_4$, NaOH, dioxane and AcOH were purchased from Panreac (Spain).

2.2. Nitrosation reaction of taurine

This reaction was monitored by measuring the absorbance of the acidic nitrosation mixtures (pH range ≈2–5). Since only the $HNO_2/NaNO_2$ system shows absorption in the UV spectrum (maximum absorption at λ = 371 nm), nitrite was used as a control species to monitor the nitrosation reaction. The nitrosation mixtures were made up by the addition of 0.1 ml of a sodium nitrite solution (concentration range 0.16–0.70 M) to a cuvette containing 3.0 ml of a taurine solution (concentration range 0.16–0.41 M), both of them made up by weight. To control ionic strength and pH, the taurine solutions contained $NaClO_4$ and $HClO_4$. No buffer solution was required because the initial rate method was to be used (Casado, López-Quintela, & Lorenzo-Barral, 1986).

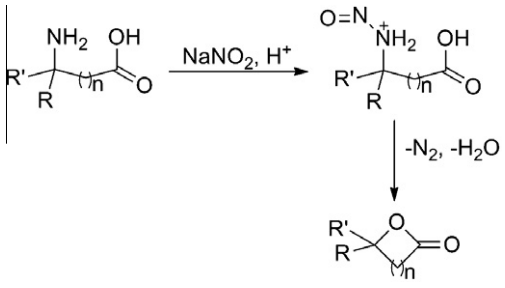

Fig. 1. Nitrosation of amino carboxylic acids with the –NH_2 group (García-Santos et al., 2001).

2.3. Alkylation reactions resulting from the nitrosation of taurine

To check the formation of a potential alkylating derivative in the nitrosation mixtures, the nucleophile 4-(*p*-nitrobenzyl)pyridine (NBP), a trap for alkylating agents with nucleophilic characteristics similar to those of DNA bases (Kim & Thomas, 1992), was used. NBP is known to react with strong (Manso, Pérez-Prior, García-Santos, Calle, & Casado, 2005) and weak (Pérez-Prior et al., 2010) alkylating agents, and much insight into such alkylation mechanisms *in vivo* can be gained from kinetic study of this reaction *in vitro* (Gómez-Bombarelli et al., 2008; Pérez-Prior et al., 2009). Since the nitrosation product ethanesultone (ES; *vide infra*) reacted with NBP, the absorbance of the adduct formed was used to monitor the alkylation reaction. The NBP test was performed as follows: 2.4-ml aliquots of the alkylation mixture were removed at different times and added to a cuvette containing 0.6 ml of 99% triethylamine (Et_3N) to form a coloured compound (see supporting information), whose absorbance was measured at λ = 541 nm (maximum absorption; see supporting information). The alkylation mixtures (performed in 7:3 water/dioxane medium owing to the insolubility of NBP in water) were made by the addition of 10.0 ml of a nitrosation mixture to 40.0 ml of NBP solution (concentration range 0.0075–0.025 M). Acetate buffer was used to maintain pH constant (pH = 5.4) in the alkylation reaction. The nitrosation mixtures were prepared at 25.0 °C and pH = 3.20 ($[nitrite]_o$ = 0.1 M; $[Tau]_o$ = 0.1 M). In order to gain a suitable $[ES]_o$ for the alkylation reaction, the nitrosation mixtures were incubated for 15 min. Detailed reaction conditions are given in the figure and table legends.

All kinetic runs were performed in triplicate.

3. Results

3.1. Nitrosation

In the investigation of the taurine nitrosation reaction, the initial rate of disappearance of nitrite, v_o, was observed to be the sum of the initial rates of nitrosation, v_N, and nitrite decomposition, v_D. The nitrosation rate equation was of second order with respect to nitrite and of first order with respect to taurine:

$$v_o = v_N + v_D = k_{obs}[Tau]_o[nitrite]_o^2 + v_D \quad (1)$$

k_{obs} being the experimental rate constant of taurine nitrosation.

No influence of ionic strength (experiments within the *I* range of 0.40–0.80 M were carried out) on the reaction rate was observed.

On the basis of previous results concerning the nitrosation of amino carboxylic acids with the –NH_2 group (García-Santos et al., 2001, 2002), the second order of the reaction with respect to nitrite observed in the present work, as well as the absence of a first order in nitrite, the mechanism shown in Fig. 2 can be proposed for the nitrosation of taurine.

From this reaction mechanism, Eq. (2) can be deduced easily:

$$v_N = k_{nit}K_MK_{II}\frac{[H^+]^2}{([H^+]+K_{II})(K_a+[H^+])^2}[Tau]_o[nitrite]_o^2 \quad (2)$$

where $K_M = K_aK_2K_3 = 3\times10^{-3}\ M^{-1}$ is the Markovits constant (Markovits, Schwartz, & Newman, 1981), $K_{II} = 10^{-9.06}$ M (Hamborg, Niederer, & Versteeg, 2007), and k_{nit} is the taurine nitrosation rate constant.

According to Eqs. (1) and (2), k_{obs} can be written as:

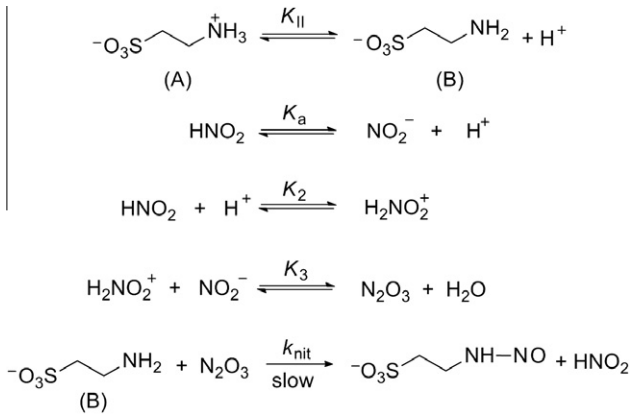

Fig. 2. Mechanism of the nitrosation of taurine.

$$k_{obs} = k_{nit}K_M K_{II}\frac{[H^+]^2}{([H^+]+K_{II})(K_a+[H^+])^2} \quad (3)$$

which transforms into Eq. (4) when $[H^+] >> K_{II}$.

$$k_{obs} = \alpha\frac{[H^+]}{(\beta+[H^+])^2} \quad (4)$$

where $\alpha = k_{Nit}K_M K_{II}$ and $\beta = K_a$.

In order to calculate the k_{obs} values as a function of pH, it is necessary to avoid interference from the decomposition reaction of nitrous acid. To do so, kinetic experiments aimed at measuring v_o (Eq. (1)) as a function of $[Tau]_o$ for different pHs (2.0–5.0 range) were performed , and the k_{obs} values were calculated from the slope depicted in Fig. 3(a) and those of v_D from the intercept. Fig. 3(b) shows the k_{obs} values with and without correction. As can be observed, the influence of nitrite decomposition is negligible at pH $\geqslant$ 3.2.

The values $\alpha = 3.21 \times 10^{-4}\ M^{-1}\ s^{-1}$ and $\beta = 10^{-3.02}$ M were obtained from fitting the experimental data to Eq. (4).

With the values of α and β, the $k_{nit} = 1.23 \times 10^8\ M^{-1}\ s^{-1}$ and $K_a = 10^{-3.02}$ M values were calculated.

The activation parameters ($\Delta^{\ddagger}H^0$ and $\Delta^{\ddagger}S^0$) for the taurine nitrosation reaction were calculated with the values of k_{obs} measured at different temperatures (17.5–40.0 °C).

The k_{obs} values (see supporting information) show a good fit to the Eyring–Wynne–Jones equation (Connors, 1990):

$$k_{obs} = \frac{kT}{h}e^{\frac{\Delta^{\ddagger}S^0}{R}}e^{\frac{-\Delta^{\ddagger}H^0}{RT}} = \frac{kT}{h}e^{\frac{-\Delta^{\ddagger}G^0}{RT}} \quad (5)$$

The values $\Delta^{\ddagger}H^0 = 55 \pm 2$ kJ mol^{-1} ($E_a = 57 \pm 2$ kJ mol^{-1}) and $\Delta^{\ddagger}S^0 = -82 \pm 1$ J mol^{-1} K^{-1} were obtained.

Since $\Delta^{\ddagger}H^0$ (observed) = ΔH^0 (formation of N_2O_3) + ΔH^0 (protonation of the taurine amine group) + $\Delta^{\ddagger}H^0$ (taurine nitrosation reaction), and because ΔH^0 (formation of N_2O_3) = 5.9 ± 0.5 kJ mol^{-1} (Casado, Castro, Leis, López-Quintela, & Mosquera, 1983) and ΔH^0 (protonation of taurine amine group) = 41.5 ± 0.1 kJ mol^{-1} (Hamborg et al., 2007), it may be deduced that $\Delta^{\ddagger}H^0$ (nitrosation) = 8 ± 2 kJ mol^{-1}.

Upon the basis of: (i) The fact that positive-mode electrospray ionisation mass spectrum of a sample from the nitrosation reaction afforded a mass/charge ratio m/z = 106.8, coherent with the structure of ethanesultone (the signal disappeared along the reaction time). This result is consistent with the formation of lactones in the nitrosation of homologous amino carboxylic acids (Williams, 2004); (ii) The fact that large lactones do not undergo hydrolysis in neutral media (Pérez-Prior, Manso, García-Santos, Calle, & Casado, 2005), as opposed to homologous sultones (Osterman-Golkar & Wachtmeister, 1976); (iii) the formation of isethionic acid (ITA) as the final product of the nitrosation of taurine (δ = 3.0 (*t*, ^{3}J (H,H) = 6.5 Hz, 2 H; CH_2), 3.8 ppm (*t*, ^{3}J (H,H) = 6.5 Hz, 2 H; CH_2) see Supporting Information), the reaction mechanism shown in Fig. 4 can be proposed, with ES as an intermediate species.

3.2. NBP alkylation

Since the NBP-ES adduct undergoes hydrolysis, a mechanism of consecutive reactions is proposed for NBP alkylation by ethanesultone (Fig. 4).

The rate equation observed for the formation of the NBP-ES adduct [AD] was:

$$v = \frac{d}{dt}[AD] = k_{alk}[NBP][ES] - k_{hyd,AD}[H_2O][AD] \quad (6)$$

k_{alk} and $k_{hyd,AD}$ being the rate constants of the NBP alkylation reaction and the hydrolysis of AD, respectively.

Rate Eq. (7) accounts for the disappearance of ethanesultone:

$$v = -\frac{d}{dt}[ES] = k_{alk}[NBP][ES] + k_{hyd,ES}[H_2O][ES] \quad (7)$$

Because NBP and water were present in large excess, their concentrations were assumed to be constant. Hence:

$$k'_{hyd,ES} = k_{hyd,ES}[H_2O] \quad k'_{alk} = k_{alk}[NBP]_o \quad (8)$$

Substitution of Eqs. (8) into Eq. (7) and integration yields Eq. (9):

$$[ES] = [ES]_o e^{-(k'_{alk}+k'_{hyd,ES})t} \quad (9)$$

Substitution of Eq. (9) into Eq. (6), and integration and substitution of [AD] by the corresponding absorbance $A_{AD} = [AD]l\varepsilon_{AD}$ (ε_{AD} being the molar absorption coefficient of the adduct and l the light path) yield Eq. (10):

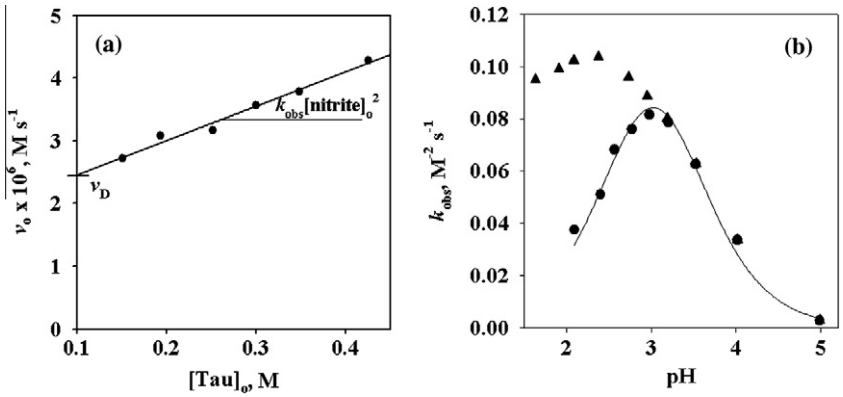

Fig. 3. (a) Variation in the initial rate of nitrosation; (b) Variation in k_{obs} with pH: (▲) Observed values; (●) corrected values without HNO_2 decomposition. $[nitrite]_o$ = 0.010 M; T = 25.0 °C; I = 0.50 M.

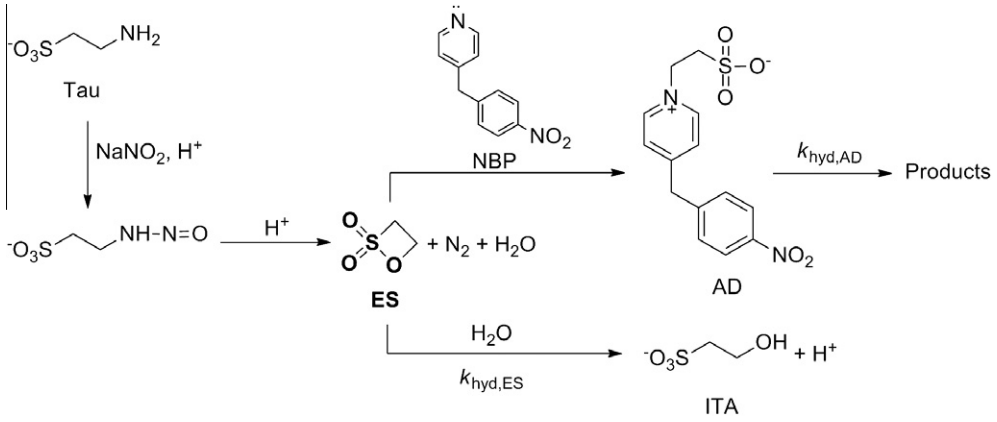

Fig. 4. Conversion of nitroso taurine into ethanesultone and isethionic acid and alkylation of NBP by ethanesultone.

$$A_{AD} = \frac{c}{b-a}(e^{-at} - e^{-bt}) \quad (10)$$

where a, b and c are defined as follows:

$$a = k'_{alk} + k'_{hyd,ES} \quad b = k'_{hyd,AD} \quad c = \varepsilon_{AD} l [ES]_o k'_{alk} \quad (11)$$

Fig. 5 shows the good fit of Eq. (10) to the results.

Table 1 shows the rate constants of NBP alkylation and the ethanesultone hydrolysis rate constants at different temperatures.

The activation parameters for the NBP alkylation and hydrolysis of ethanesultone were also calculated. Their values are shown in Table 1.

The fraction, f, of ES forming the adduct with NBP (Eq. (12)), as well as the adduct life, AL (Eq. (13)), and the total amount of adduct present along the progression of the reaction per unit of alkylation agent concentration, (Pérez-Prior et al., 2009) were also calculated:

$$f = \frac{k_{alk}[NBP]}{k_{alk}[NBP] + k_{hyd,ES}[H_2O]} \quad (12)$$

$$AL = \frac{\int_0^\infty [AD]dt}{[ES]_o} = \frac{k_{alk}[NBP]}{(k_{alk}[NBP] + k'_{hyd,ES})k'_{hyd,AD}} = \frac{f}{k'_{hyd,AD}} \quad (13)$$

The f and AL values are given in Table 1.

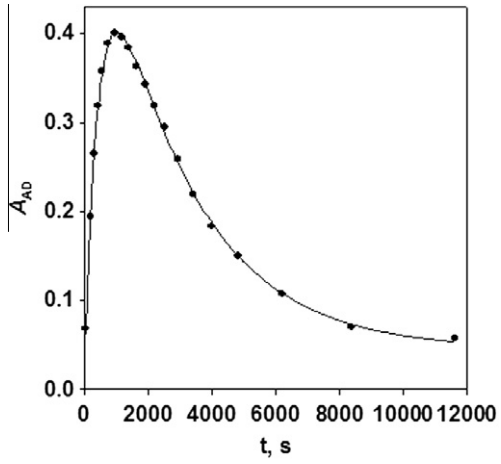

Fig. 5. Fit of the absorbance of the NBP-ES adduct along time to Eq. (10). $[NBP]_o = 0.012$ M; $T = 20.0$ °C; water/dioxane (7:3); pH = 5.4; $t_{nit} = 15$ min; $\lambda = 541$ nm.

4. Discussion

As stated above, the experimental results suggested that N_2O_3 was the effective nitrosating agent. The finding that the nitrosation rate was proportional to the square of the nitrite concentration suggests that the yield of nitrosation products in the human stomach would increase sharply with higher nitrate/nitrite intakes.

The values $k_{nit} = 1.23 \times 10^8$ M^{-1} s^{-1} and $\Delta^{\ddagger}H^{\theta}$ (nitrosation) = 8 ± 2 kJ mol^{-1} (*vide supra*) suggest that the nitrosation reaction of taurine is controlled by diffusion (Ridd, 1978). This is consistent with previous studies addressing the reactivity of N_2O_3 as a nitrosating agent (Casado et al., 1983) and supports the mechanism proposed here, with dinitrogen trioxide as the effective nitrosating agent. A comparison of the reactivity of taurine ($k_{nit} = 1.23 \times 10^8$ M^{-1} s^{-1}) with that of amino carboxylic acids with the $-NH_2$ group shows that β-alanine (the homologous amino acid of taurine) is four times more reactive than taurine ($k_{nit} = 4.40 \times 10^8$ M^{-1} s^{-1}). Only the rate constant for the nitrosation of alanine is lower ($k_{nit} = 0.60 \times 10^8$ M^{-1} s^{-1}) (García-Santos et al., 2002). This result can be rationalised in terms of the smaller electron-donating (I+) effect of the SO_3^- group at the nucleophilic site of the taurine molecule.

These results could be of interest in food science as well as for environmental chemistry. Among others, two aspects on deserving attention are:

(i) Taurine occurs naturally in food, especially in seafood and meat. Table 2 shows comparative contents of this compound in several foods. Taurine is also regularly used as an ingredient in some energy drinks and nutraceuticals, many of them containing 1000 mg per serving, and some as much as 2000 mg.

Since many of the most potent carcinogens are included among *N*-nitroso compounds (Lijinsky, 1992), and since some of their derivatives, such as β-lactones, have been classified as possibly carcinogenic to humans (Lijinsky, 1992), the results found here suggest that ES would be a potentially mutagenic agent.

(ii) The NBP alkylation rate constant for ethanesultone at 25 °C ($k_{alk} = 8.52$ M^{-1} min^{-1}) is much higher than those of BPL and BBL, $k_{alk} = 0.47$ M^{-1} min^{-1} and $k_{alk} = 0.04$ M^{-1} min^{-1} respectively. However, the fraction, f, of ES forming the adduct with NBP ($f = 0.79$) is similar to that of BBL ($f = 0.83$) and BPL ($f = 0.72$) because the hydrolysis rate constant of ES is also much higher ($k_{hyd,ES} = 114.8 \times 10^{-5}$ M^{-1} min^{-1} vs. $k_{hyd,BPL} = 4.88 \times 10^{-5}$ M^{-1} min^{-1} and $k_{hyd,BBL} = 0.81 \times 10^{-5}$ M^{-1} min^{-1}) (Manso et al., 2005). In addition, the adduct life ($AL_{ES} = 13.60$ min) is very short because the adduct formed by ES with NBP is not stable, while for BPL and BBL the adduct is stable. A similar situation to ES occurs with lactone diketene (Gómez-Bombarelli et al., 2008).

Table 1
Rate constants and activation parameters for the NBP alkylation and ES hydrolysis reactions. $[NBP]_o$ = 0.020–0.006 M; Water/dioxane 7:3; pH = 5.4.

T (°C)	NBP alkylation k_{alk} (M^{-1} s^{-1})[b]	ES hydrolysis $k_{hyd,ES} \times 10^5$ (M^{-1} s^{-1})[b]	f[c]	AL[c] (min)
15.0	0.1085	0.900	0.86	64.6
17.5	0.1195	1.072	0.85	47.0
20.0	0.1260	1.396	0.82	30.6
22.5	0.1301	1.618	0.81	20.6
25.0	0.1420	1.913	0.79	13.6
37.5	–	–	0.70[a]	1.97[a]
$\Delta^{\ddagger}H^{0}$ (kJ mol^{-1})	15 ± 2	52 ± 3		
$\Delta^{\ddagger}S^{0}$ (J K^{-1} mol^{-1})	−210 ± 6	−159 ± 10		
$\Delta^{\ddagger}G^{0}$ (35 °C) (kJ mol^{-1})	80 ± 3	101 ± 4		

[a] Values extrapolated from the fit of f values to temperature.
[b] Values are reproducible to 3%.
[c] $[NBP]_o$ = 0.020 M.

Table 2
Content of taurine in some foods.

Food	Taurine content (mg/250 g)
Chickpeas[a]	1.1
Beef meat[b]	192
Albacore tuna[c]	440
Energy drinks	1000
Clams[d]	1295
Octopus[d]	976
Shrimp[d]	388

[a] From (Pasantes-Morales & Flores, 1991).
[b] From (Purchas, Rutherfurd, Pearce, Vather, & Wilkinson, 2004).
[c] From (Gormley, Neumann, Fagan, & Brunton, 2007).
[d] From (Pasantes-Morales, Quesada, Alcocer, & Olea, 1989).

As can be seen from Table 1, the values of f and AL decrease with temperature. At physiological temperature (≈37.5 °C), the alkylating capacity of ethanesultone is still high but its alkylating efficiency is low. Due to this, ethanesultone can be expected to have low activity as a mutagenic species.

In spite of this low risk to human health, the stomach lumen conditions (pH ≈ 3 during the digestion process) could be a favourable medium for the nitrosation of taurine and hence the formation of the alkylating ethanesultone. Thus, more attention should be paid to potential situations of the concurrence of high contents of taurine and nitrite/nitrate in the diet.

5. Conclusions

The conclusions drawn are as follows:

(i) Nitrite shows nitrosating capacity on the taurine molecule. The rate equation was $v_N = k_{obs}[Tau]_o[nitrite]_o^2$, suggesting that the yield of nitrosation products in the human stomach would increase sharply with higher nitrate/nitrite intakes.
(ii) The experimental results suggest a mechanism for the nitrosation, whose rate-limiting step is bimolecular attack by N_2O_3 on the free-base form of the amino group.
(iii) The nitrosation of taurine affords ethanesultone (ES), which exerts alkylating capacity on the nucleophile 4-(*p*-nitrobenzyl)pyridine (NBP), a trap for alkylating agents with nucleophilic characteristics similar to those of DNA bases. Although the NBP alkylation rate for ethanesultone is much higher than those for carcinogenic four-membered ring lactones resulting from the nitrosation of amino carboxylic acids (viz. β-propiolactone, formed from the nitrosation of β-alanine, the homologous amino acid of taurine), the fraction of ES forming an adduct with NBP is much smaller because the hydrolysis rate of ES is also much higher. Because of this, and also due to the short life of the ES-NBP adduct, ES can be expected to have low activity as a mutagenic species.
(iv) In spite of the low risk to human health, since the stomach lumen conditions could be a favourable medium for the nitrosation of taurine and hence the formation of the alkylating ES more attention should be paid to potential situations of the concurrence of high contents of taurine and nitrite/nitrate in the diet. Since the observed nitrosation rate constant shows a maximum for pH = 3.2, stomach hypochlorhydria could be a potential enhancer of taurine nitrosation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2012.03.005.

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