

Sorbic Acid as an Alkylating Agent

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Abstract A kinetic study of the alkylating potential of the sorbic acid + NaOH and sorbic acid + KOH systems was performed in 7:3 (volume/volume) water + dioxane solvent mixtures. The following conclusions were drawn. First, the sorbic acid + sorbate system shows alkylating activity on the nucleophile 4-(*p*-nitrobenzyl)pyridine (NBP), which is used as a trap for alkylating agents having nucleophilic characteristics similar to DNA bases. Second, the maximum alkylating capacity is observed in the pH = 5.0 to 6.5 range. Third, the alkylation reactions comply with the rate equation $r = k_{\text{alk}}[\text{H}^+][\text{S}][\text{NBP}]/(K_a + [\text{H}^+])$, with K_a being the dissociation constant of sorbic acid. Fourth, an enthalpy–entropy ($\Delta H^\ddagger/\Delta S^\ddagger$) compensation effect for activation quantities is observed by comparing NBP alkylation reactions due to sorbic acid + NaOH, sorbic acid + KOH, as well as potassium sorbate + HCl mixtures. Fifth, the results may help to establish suitable expiration times for products preserved with sorbic acid.

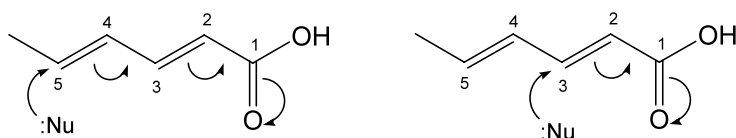
Keywords Sorbic acid · Alkylating potential · Food preservatives

1 Introduction

Sorbic acid and its salts are used as preservatives in a large number of foods, feeds, pharmaceuticals, cosmetics and packaging material. The antimicrobial properties of sorbic acid were discovered independently in 1939 and 1940 by Müller and Gooding [1] (in Germany and the USA, respectively). After this discovery, sorbic acid and its salts were tested and used in a variety of consumer products for the inhibition of growth of yeast and molds, and for control of certain bacteria [2].

Sorbates have been reported to be more efficient and less toxic than benzoate [1] and are classified as ‘Generally Recognized as Safe’ (GRAS) additives by the FDA [3]. In the European Union, the use of sorbic acid (E200) and its potassium (E202) and calcium (E203) salts

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Scheme 1 Nucleophilic attack to the molecule of sorbic acid

is authorized to lengthen the shelf life of many foods (including margarine, fruit preserves and desserts, wines and other drinks, moist cheeses, etc.) [4].

Sorbic acid and potassium sorbate are less genotoxic than sodium sorbate [5], the two forms that are considered as GRAS. Significant data [6] indicate there is a weak genotoxic potential for stored sodium sorbate solutions. Accordingly, sodium sorbate is no longer in use as a food preservative and is not commercially available [7].

Sorbic acid has a conjugated system of double bonds that renders it susceptible to nucleophilic attack by species such as thiols [8, 9], amines [10] and nitrites [11, 12], sometimes producing mutagenic products [4]. Strong nucleophiles, such as amines and thiols, yield 1,4-addition products without the need for an initial protonation of the sorbate, the addition of H^+ taking place as the final stage in the mechanism [9]. The much greater extent to which the charge on the intermediate is delocalized (arising from an attack at position 5 of sorbic acid) suggests that this position should be preferred, despite the lower electron density at position 3 (see Scheme 1).

The nucleophile NBP, 4-(*p*-nitrobenzyl)pyridine, which is a trapping molecule for alkylating agents [13] with nucleophilic characteristics similar to DNA bases [14], has previously been used by us to measure the alkylating potential of lactones [15–17]. A correlation was found between their alkylating capacity and carcinogenicity [18].

Although many aspects related to the worldwide use of sorbic acid and its salts as food preservatives have long been known, there is little quantitative knowledge about their alkylating potential.

In our previous work the alkylating potential of potassium sorbate in aqueous hydrochloric acid conditions was studied [19]. In the present study, the alkylation of NBP by sorbic acid + KOH and sorbic acid + NaOH mixtures was investigated and the results are compared.

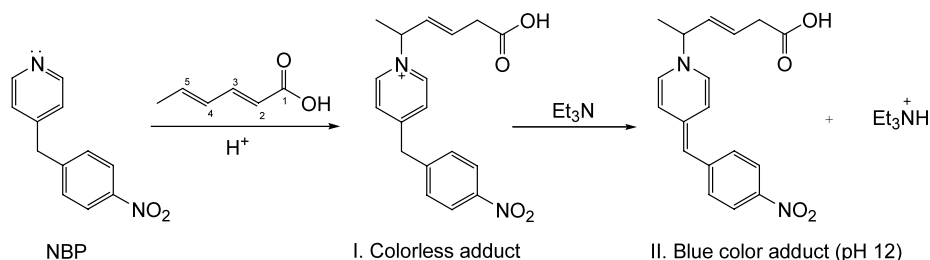
2 Experimental

2.1 Materials

NBP was from Sigma; sorbic acid, potassium hydroxide and dioxane were purchased from Panreac (Barcelona, Spain); sodium hydroxide was from Fluka, and Et_3N (99%) was obtained from Aldrich. Water was deionized with a Milli Q-Gradient (Millipore) system.

A Shimadzu UV-2401-PC spectrophotometer with a thermoelectric six-cell holder temperature control system ($\pm 0.1^\circ C$) was used. A Crison Micro pH 2000 pH meter with a 5202 electrode was used for the pH measurements [20].

The reaction temperature was kept constant ($\pm 0.05^\circ C$) with a Lauda Ecoline RE120 thermostat. All kinetic runs were performed in triplicate.



Scheme 2 Method for monitoring the NBP alkylation by sorbate

2.2 Methods

Alkylation reactions of sorbate *S* (expressed as the sum of the concentrations of undissociated sorbic acid *AH*, plus that present as the sorbate anion A^-) were carried out at constant pH over the range $\text{pH} = 5.0$ to 6.5 that is frequently found in foods [2], e.g., bread: $\text{pH} = 5.0$ to 6.0 ; chicken: $\text{pH} = 5.5$ to 6.4 ; cheese: $\text{pH} = 5.0$ to 6.1 ; beef: $\text{pH} = 5.3$ to 6.2 ; etc. The $S + \text{NaOH}$ and $S + \text{KOH}$ systems act as buffers, keeping the pH constant. In order to make NBP sufficiently soluble, the alkylation mixtures ($S + \text{NBP} + \text{NaOH}$ or KOH) were prepared in a 7:3 (volume/volume) water + dioxane medium.

To monitor the alkylation reactions, 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 reagent (Et_3N) to terminate the alkylation process (Scheme 2). Then, the absorbance was measured at the wavelength of maximum absorption ($\lambda = 580$ nm). Detailed reaction conditions are given in the figures and tables legends.

3 Results and Discussions

3.1 Kinetics of the Alkylation Reaction

The blue-colored sorbate + NBP adduct showed a maximum absorption at $\lambda = 580$ nm. For example, Fig. 1 shows the increase in absorption caused by formation of the adduct over time, until no further change in absorbance (*A*) was observed. Because the sorbate concentration was in large excess as compared with NBP, it may be assumed that all of the NBP was converted into the adduct (AD).

At $\text{pH} \leq 5$, the alkylation reaction cannot be followed because crystals of sorbic acid begin to precipitate (the solubility of sorbic acid is 0.16% in water at 25°C whereas those of its potassium and sodium salts are over 138 g and 20 g per 100 mL water, respectively) [1, 2]. Figure 2 represents a typical kinetic run for the alkylation of NBP by sorbic acid in the $S + \text{NBP} + \text{KOH}$ alkylation mixture.

Because (i) the reactivity of sorbate increases with decreasing pH, and (ii) the antimicrobial activity of the organic acids and their salts is mainly attributed to the inhibiting action of the undissociated acid molecule [2, 21, 22], in a previous work the reaction mechanism shown in Scheme 3 was proposed by us [19], where K_a is the sorbic acid dissociation constant and k_{alk} is the NBP alkylation rate constant for the undissociated acid.

Because

$$[S] = [AH] + [A^-] \quad (1)$$

Fig. 1 Spectrograms showing the formation of the sorbate + NBP adduct concentration over time in 7:3 (volume/volume) water + dioxane mixtures, obtained as the variation of the absorbance from 1 to 13 days.

Conditions:

$[S]_0 = 0.15 \text{ mol}\cdot\text{L}^{-1}$, $[\text{KOH}] =$

$0.1 \text{ mol}\cdot\text{L}^{-1}$,

$[\text{NBP}]_0 = 0.003 \text{ mol}\cdot\text{L}^{-1}$, $\text{pH} =$

5.78, and $t = 35.0^\circ\text{C}$

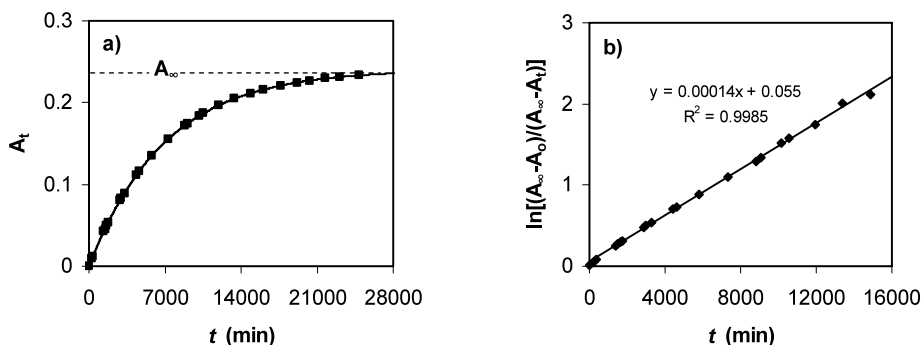
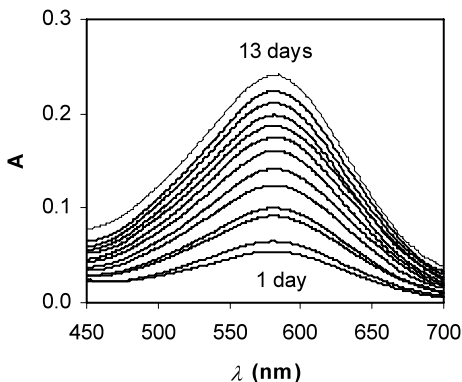
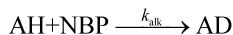
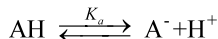


Fig. 2 (a) Formation of the sorbate + NBP adduct in 7:3 (volume/volume) water + dioxane mixtures. Variation in the absorbance ($\lambda = 580 \text{ nm}$) with time, with A_∞ being the absorbance of the adduct when all the NBP has been consumed. (b) Determination of the NBP alkylation pseudo-first-order rate constant (k_1 in Eq. 7) by sorbate in 7:3 (volume/volume) water + dioxane mixtures. Conditions: $[S]_0 = 0.15 \text{ mol}\cdot\text{L}^{-1}$, $[\text{KOH}] = 0.1 \text{ mol}\cdot\text{L}^{-1}$, $[\text{NBP}]_0 = 0.003 \text{ mol}\cdot\text{L}^{-1}$, $\text{pH} = 5.78$, and $t = 35.0^\circ\text{C}$

Scheme 3 NBP alkylation by sorbate



and $K_a = ([\text{A}^-][\text{H}^+])/[\text{AH}]$, Eq. 2 is easily deduced from Scheme 3 and Eq. 1:

$$\text{rate} = \frac{dx}{dt} = k_{\text{alk}} \frac{[\text{H}^+]}{K_a + [\text{H}^+]} [\text{S}][\text{NBP}]. \quad (2)$$

We designate the fraction of sorbate converted into adduct by the alkylation reaction as x , let ε_{NBP} and ε_{AD} denote the molar absorption coefficients of NBP and AD, respectively, and let A_0 , A_t , A_∞ represent the initial absorbance, the absorbance at time t , and the final absorbance, respectively. Equations 3 to 5 can then be written as:

$$A_0 = \varepsilon_{\text{NBP}}[\text{NBP}]_0, \quad (3)$$

$$A_t = \varepsilon_{\text{NBP}}([\text{NBP}]_0 - x) + x\varepsilon_{\text{AD}}, \quad (4)$$

Table 1 Effect of pH on the NBP alkylation reaction for the sorbic acid + KOH and sorbic acid + NaOH systems in 7:3 (volume/volume) water + dioxane solvent mixtures. Conditions: $[\text{NBP}]_0 = 0.003 \text{ mol}\cdot\text{L}^{-1}$, $[\text{KOH}] = [\text{NaOH}] = 0.1 \text{ mol}\cdot\text{L}^{-1}$, and $t = 35.0^\circ\text{C}$

AH + NBP + KOH			AH + NBP + NaOH		
pH	[S] ($\text{mol}\cdot\text{L}^{-1}$)	$k_1 \times 10^{4a}$ (min^{-1})	pH	[S] ($\text{mol}\cdot\text{L}^{-1}$)	$k_1 \times 10^{4a}$ (min^{-1})
–	–	–	5.46	0.20	2.79 ± 0.04
5.59	0.19	2.96 ± 0.04	5.59	0.19	2.32 ± 0.03
5.65	0.18	2.35 ± 0.02	5.65	0.18	1.94 ± 0.03
5.78	0.15	1.44 ± 0.01	5.80	0.15	1.26 ± 0.01
5.89	0.14	1.07 ± 0.02	–	–	–
–	–	–	6.05	0.13	0.53 ± 0.02
–	–	–	6.32	0.11	0.37 ± 0.01

^aValues of the pseudo-first-order rate constant are given with their standard deviations

$$A_\infty = \varepsilon_{\text{AD}}[\text{NBP}]_0. \quad (5)$$

Because the pH was kept constant (see Methods), and the experiments were performed with [S] in large excess, Eq. 2 can be rewritten in the form:

$$\text{rate} = \frac{dx}{dt} = k_1([\text{NBP}]_0 - x) \quad (6)$$

where k_1 , the pseudo-first-order rate constant, is given by

$$k_1 = k_{\text{alk}}[\text{H}^+][\text{S}]/(K_a + [\text{H}^+]). \quad (7)$$

Integration of Eq. 6 and expressing the result in terms of the absorbance yields Eq. 8:

$$k_1 t = \ln \frac{A_\infty - A_0}{A_\infty - A_t} \quad (8)$$

where A_∞ is the absorbance of the adduct when the plateau is reached (Fig. 2a). Figure 2b shows the fit of the results to Eq. 8.

In order to check the possibility that a fraction of the NBP reagent was protonated, its $\text{p}K_a$ in 7:3 (volume/volume) water + dioxane solvent mixtures was determined. Measurements carried out at $\lambda = 261 \text{ nm}$, where the maximum absorbance occurs, yielded $\text{p}K_a = 4.4$. Thus, significant protonation of NBP was ruled out.

Several experiments in the pH = 5.0 to 6.5 range were carried out for the S + KOH and S + NaOH systems. Table 1 shows the resulting variation in the pseudo-first-order rate constant values, k_1 , with pH.

To check the validity of Eq. 7, the k_1 values were plotted against those of $[\text{H}^+][\text{S}]/(K_a + [\text{H}^+])$. Figure 3 shows the results, with k_{alk} being the obtained slope of the line.

The values of k_{alk} at different temperatures are shown in Table 2. Table 3 shows the activation parameters for NBP alkylation in the sorbate + NaOH and sorbate + KOH systems, as well as previous the results obtained in a hydrochloric acid medium [19]. Figure 4 depicts the good fit of the results by the Eyring equation [23]:

$$k = \frac{kT}{h} e^{\Delta S^\ddagger/R} e^{-\Delta H^\ddagger/RT} = \frac{kT}{h} e^{-\Delta G^\ddagger/RT}.$$

Fig. 3 Variation of k_1 with $[\text{H}^+][\text{S}]/(K_a + [\text{H}^+])$ (Eq. 7) in the 7:3 (volume/volume) water + dioxane solvent mixture for the AH + NBP + NaOH system. Conditions: $[\text{NaOH}] = 0.1 \text{ mol}\cdot\text{L}^{-1}$, $[\text{NBP}]_0 = 0.003 \text{ mol}\cdot\text{L}^{-1}$, $K_a = 1.8 \times 10^{-5}$, and $t = 35.0^\circ\text{C}$

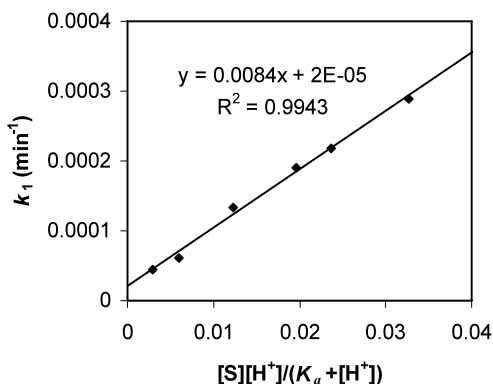


Fig. 4 Eyring plot for NBP alkylation by the sorbic acid + KOH system in 7:3 (volume/volume) water + dioxane solvent mixtures

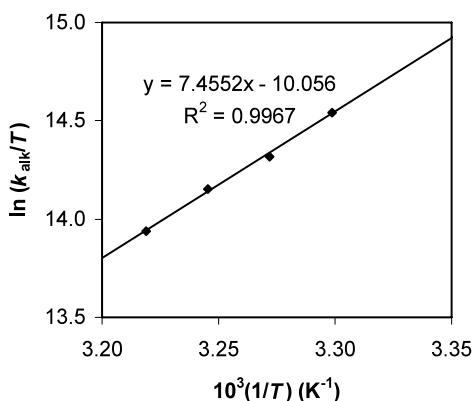


Table 2 Rate constant as a function of temperature for NBP alkylation by the sorbic acid + KOH and sorbic acid + NaOH systems in 7:3 (volume/volume) water + dioxane solvent mixtures. Conditions: $[\text{NBP}]_0 = 0.003 \text{ mol}\cdot\text{L}^{-1}$, and $[\text{KOH}] = [\text{NaOH}] = 0.1 \text{ mol}\cdot\text{L}^{-1}$

$t \text{ (}^\circ\text{C)}$	$k_{\text{alk}} \times 10^{4a} \text{ (L}\cdot\text{mol}^{-1}\cdot\text{s}^{-1}\text{)}$	
	AH + NBP + KOH	AH + NBP + NaOH
30.0	1.46 ± 0.10	1.30 ± 0.04
32.5	1.85 ± 0.06	1.34 ± 0.07
35.0	2.20 ± 0.12	1.40 ± 0.05
37.5	2.75 ± 0.07	1.50 ± 0.03

^aValues of the rate constant are given with their standard deviations

The results obtained for the alkylation of NBP by sorbic acid + NaOH and sorbic acid + KOH, as well as by potassium sorbate + HCl, show that the activation parameters fluctuate over a wide range of values. The existence of a good $\Delta H^\ddagger/\Delta S^\ddagger$ isokinetic relationship (Fig. 5) supports the argument that all the reactions share a common mechanism [24]; this is logical because the active alkylating agent is always the same (sorbic acid) in the sorbic acid + sorbate mixtures studied here. Nevertheless, because the K^+ and Na^+ ions do not participate in any of the mechanism steps (Scheme 3), more experimental work is necessary

Table 3 Activation parameters for NBP alkylation by sorbic acid + sorbate in the 7:3 (volume/volume) water + dioxane solvent mixtures

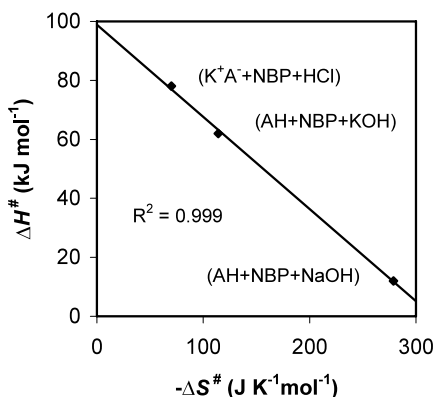
Alkylation mixture	$\Delta H^{\#a}$ (kJ·mol ⁻¹)	$-\Delta S^{\#a}$ (J·K ⁻¹ ·mol ⁻¹)
AH + NBP + KOH ^b	62 ± 3	114 ± 11
AH + NBP + NaOH ^b	12 ± 2	279 ± 20
K ⁺ A ⁻ + NBP + HCl ^c	78 ± 7	70 ± 12

^aValues are given with their standard deviations

^bThis work

^cValues taken from Ref. [19]

Fig. 5 Isokinetic relationship for NBP alkylation by the sorbic acid + sorbate system in the 7:3 (volume/volume) water + dioxane solvent mixtures



to rationalize the large differences observed for the $\Delta H^{\#}/\Delta S^{\#}$ values (Table 3). Possible causes of this difference could be either different values for the K_a constant for the AH + NaOH and AH + KOH systems or a strong salt-specific (cation) effect.

4 Conclusions

First, sorbic acid + sorbate mixtures show alkylating activity on the nucleophile 4-(*p*-nitro benzyl)pyridine (NBP), which is used as a trap for alkylating agents with nucleophilic characteristics similar to DNA bases. Second, the maximum alkylating capacity is observed in the pH = 5.0 to 6.5 range. Third, the alkylation reactions comply with the rate equation $r = k_{\text{alk}}[\text{H}^+][\text{S}][\text{NBP}]/(K_a + [\text{H}^+])$, with K_a being the sorbic acid dissociation constant. Fourth, a $\Delta H^{\#}/\Delta S^{\#}$ compensation effect is observed by comparing the NBP alkylation reactions due to sorbic acid + NaOH and sorbic acid + KOH, as well as for the potassium sorbate + HCl systems. Fifth, the results may help to establish suitable expiration times for products preserved with sorbic acid.

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