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Alkylating Potential of Potassium Sorbate

M. TERESA PÉREZ-PRIOR, JOSÉ A. MANSO, M. DEL PILAR GARCÍA-SANTOS, EMILIO CALLE, AND JULIO CASADO*

Departamento de Química física, Universidad de Salamanca, E-37008 Salamanca, Spain

A kinetic study of the alkylating potential of potassium sorbate (S)—a food preservative used worldwide—in 7:3 water/dioxane medium was performed. The following conclusions were drawn: (i) Potassium sorbate shows alkylating activity on the nucleophile 4-(*p*-nitrobenzyl)pyridine (NBP), a trap for alkylating agents with nucleophilic characteristics similar to those of DNA bases, (ii) The NBP alkylation reaction complies with the rate equation $r = k_{alk}[H^+][S][NBP]/(K_a + [H^+])$, K_a being the sorbic acid dissociation constant and k_{alk} the rate constant of NBP alkylation by the undissociated acid. In the range of pH 5−6, the alkylation time ranges between 18 days (pH 5.2) and >1 month (pH ≥6). (iii) NBP alkylation occurs through a reaction with $\Delta H^{\#} = 78$ kJ mol^{-1}, which is much higher than those of NBP alkylation by stronger alkylating agents. (iv) The absorption coefficient of the sorbate−NBP adduct was determined to be $\epsilon = 204$ M^{-1} cm^{-1} ($\lambda = 580$ nm), this value being rationalized in terms of the adduct structure. (v) The results can help to establish suitable expiration times for products preserved with potassium sorbate.

KEYWORDS: Potassium sorbate; alkylation; alkylating reactions

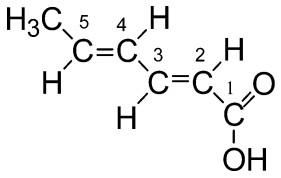
INTRODUCTION

Sorbic acid and its salts (collectively known as sorbates) have antibacterial activities and also inhibit the growth of yeasts and other fungi. Since the 1940s they have been found to be effective antimicrobial agents and hence are used as preservatives in a wide range of food and drinks and, to a lesser extent, in certain cosmetics, pharmaceuticals, and tobacco products. The range includes foods such as cheese products, pickles, certain fish products, carbonated beverages, margarine, and certain fruit and vegetable products, including wines (*1*, *2*).

Sorbates are reported to be more efficient and less toxic than benzoate (*1*) and are classified as "Generally Recognized as Safe" (GRAS) additives by the U.S. FDA (*3*). Nevertheless, because a weak genotoxic potential of stored sorbate solutions has been reported (*4*, *5*), this species is practically no longer in use in the food industry and is not commercially available. In the European Union, the use of sorbic acid and its potassium and calcium salts is authorized in many foods to lengthen their shelf life (*6*).

There is no indication that potassium sorbate (S) or its breakdown products are carcinogenic. However, there is evidence of cytotoxicity. One study showed that rats exposed to potassium sorbate had detectable cell injury. The injury levels were reduced by the introduction of an antioxidant (*7*).

Sorbic acid (structure shown below), the *trans-trans* form of hexa-2,4-dienoic acid having a π-structure with two conjugate double bonds, is likely to undergo reactions with nucleophiles



such as amines (*6*, *8*−*10*), thiols (*11*), and nitrites (*12*), which may turn it into mutagenic products (*6*).

Nucleophiles such as amines and thiols give 1,4-addition products in their reactions with dienes without the need for initial protonation, the addition of H^+ taking place as the final stage in the mechanism (*13*). This means that a nucleophile will attack the molecule of sorbic acid at positions 3 and 5. The much greater extent to which the charge on the intermediate arising from attack at position 5 is delocalized (see **Scheme 1**) suggests that this should be preferred. Steric considerations, based on the large size of the NBP molecule, also support this preference.

The nucleophile NBP, 4-(*p*-nitrobenzyl)pyridine, a trap for alkylating agents (*14*) with nucleophilic characteristics similar to those of DNA bases (*15*), was previously used by us to measure the alkylating potential of lactones. A correlation was found between alkylating capacity and carcinogenicity (*16*−*19*).

Although many aspects related to the worldwide use of potassium sorbate as a food preservative have long been known, to our knowledge the alkylating potential of this molecule has not been investigated in quantitative chemical terms. The present study addresses this aim.

* Author to whom correspondence should be addressed (e-mail jucali@usal.es; telephone +34 923 294486; fax +34 923 294574).

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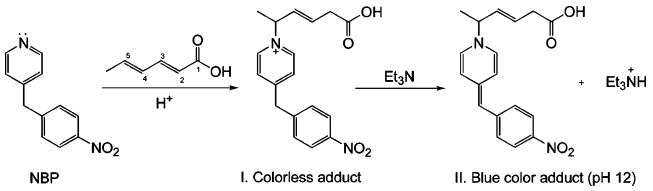
Scheme 1. Method for Monitoring the NBP Alkylation by Sorbate



Scheme 2. NBP Alkylation by Sorbate

$$AH \underset{}{\overset{K_a}{\rightleftharpoons}} A^- + H^+$$

$$AH + NBP \xrightarrow{k_{alk}} AD$$

MATERIALS AND METHODS

Alkylation reactions by potassium sorbate were carried out in aqueous hydrochloric acid conditions [pH 5−6 range, frequent in foods (*2*), for example, bread, 5.0−6.0; chicken, 5.5−6.4; cheese, 5.0−6.1; beef, 5.3−6.2]. To render NBP soluble, the alkylation mixtures (S + NBP + HCl) were prepared in 7:3 (v/v) water/dioxane medium (the system S + HCl acts as a buffer, keeping pH constant).

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 stop the alkylation process (**Scheme 1**), and then the absorbance at the wavelength of maximum absorption (λ = 580 nm) was measured. Detailed reaction conditions are given in the figure captions.

A Shimadzu UV-2401-PC spectrophotometer with a thermoelectric six-cell holder temperature control system (±0.1 °C) was used.

A Crison Micro pH 2000 pH-meter with a 5202 electrode was used to make the pH measurements (*20*).

The reaction temperature was kept constant (±0.05 °C) with a Lauda Ecoline RE120 thermostat.

All kinetic runs were performed in triplicate.

Potassium sorbate and NBP were Sigma products; 99% Et_3N was obtained from Aldrich; hydrochloric acid and dioxane were purchased from Panreac (Barcelona, Spain).

Geometry optimization of the sorbate−NBP adduct was carried out with the ChemBats3D Ultra Molecular Modeling and Analysis software, version 8.0.

RESULTS AND DISCUSSION

Kinetics of the Alkylation Reaction. The blue adduct sorbate−NBP shows maximum absorption at λ = 580 nm. As an example, **Figure 1** shows the increase in absorption caused by the formation of the adduct with time, until no change in absorbance *A* was observed. Because [S] was in large excess

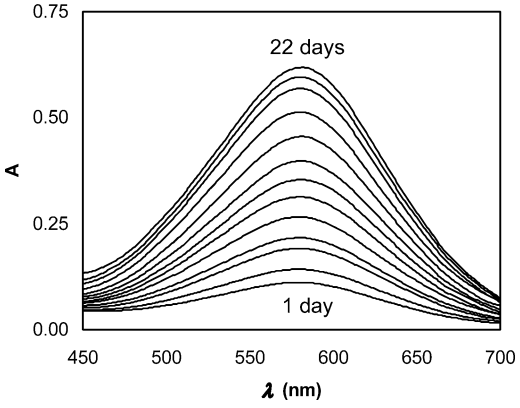


Figure 1. Spectrograms showing the formation of the sorbate–NBP adduct with time in 7:3 water/dioxane medium and variation in absorbance over the 1–22 day interval. $[S]_0$ = 0.2 M; [HCl] = 0.1 M; $[NBP]_0$ = 0.003 M; pH 5.5; *T* = 35 °C.

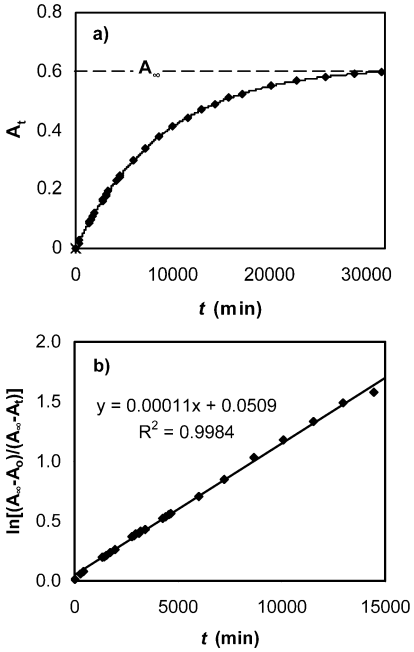


Figure 2. (**a**) Formation of the sorbate–NBP adduct in 7:3 water/dioxane medium; variation in absorbance (λ = 580 nm) with time, A_∞ being the absorbance of the adduct when all of the NBP has been consumed. (**b**) Determination of the NBP alkylation pseudo-first-order rate constant (as k_1 in eq 7) by sorbate in 7:3 water/dioxane medium. $[NBP]_0$ = 0.003 M; $[S]_0$ = 0.2 M; [HCl] = 0.1 M; pH 5.5; *T* = 35 °C.

compared with [NBP], it may be assumed that all of the NBP was converted into adduct (AD).

Whereas at pH 5.2 the time required for the alkylation plateau to be reached (see **Figure 2a**) is ∼18 days, at pH ≥6 this time is >1 month. At pH ≤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 that of its potassium salt is >50%) (*1*, *2*).

Figure 2 represents a typical kinetic run for the alkylation of NBP by potassium sorbate. On the basis of (i) the fact that 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*), the reaction mechanism shown in **Scheme 2** is proposed.

To check the possibility of a fraction of the reagent NBP being protonated, its pK_a in 7:3 water dioxane medium was determined. Measurements carried out at λ = 261 nm (an isosbestic point was observed at λ = 275 nm), where a maximum absorbance appears, gave pK_a = 4.4. Thus, significant protonation of NBP was disregarded.

The sorbate concentration [S] can be expressed as the sum of the concentration of undissociated sorbic acid (AH) plus that present as sorbate anion (A^-):

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

Because $K_a = ([A^-][H^+])/[AH]$, eq 2 is easily deduced from **Scheme 2** and eq 1:

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

By designating the fraction of potassium sorbate converted into adduct in the alkylation reaction as *x*, ϵ_{NBP} and ϵ_{AD} representing the molar absorption coefficients of NBP and AD,

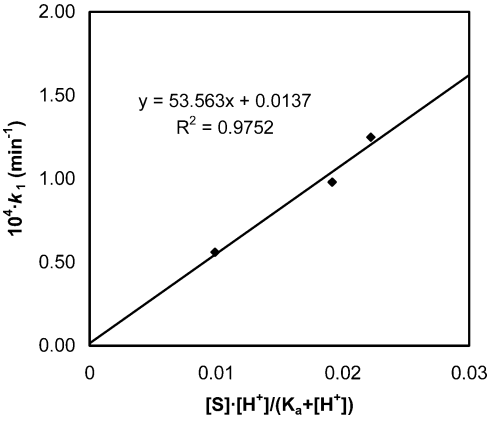


Figure 3. Variation of k_1 with $[H^+][S]/(K_a + [H^+])$ (eq 7) in 7:3 water/dioxane medium. $[NBP]_0 = 0.003$ M; $K_a = 1.8 \times 10^{-5}$; $T = 35$ °C.

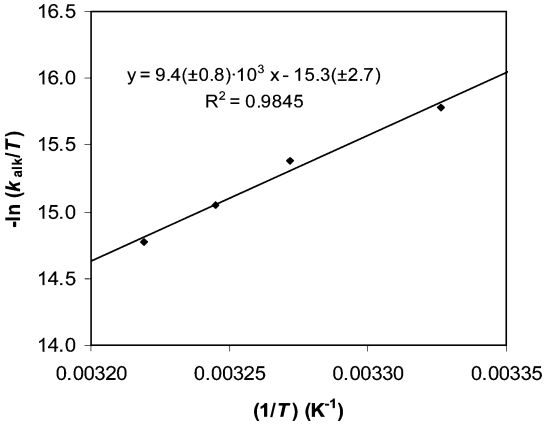


Figure 4. Eyring plot for the alkylation of NBP by potassium sorbate in 7:3 water/dioxane medium.

respectively, and A_0, A_t, A_∞ being the initial absorbance, the absorbance at time t, and the final absorbance, respectively, eqs 3−5 can be written.

$$A_0 = \epsilon_{NBP}[NBP]_0 \quad (3)$$

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

$$A_\infty = \epsilon_{AD}\,[NBP]_0 \quad (5)$$

Because the pH is kept constant (see Materials and Methods) and the experiments were performed with [S] in large excess, eq 2 can be written in the form

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

k_1, the pseudo-first-order rate constant, being

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

Integration of eq 6 and expression of the result in terms of absorbance yield eq 8

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

where A_∞ is the absorbance of the adduct when the plateau is reached (**Figure 2a**).

Figure 2b shows the good fit of the results to eq 8.

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

Figure 4 depicts the good fit of the results to the Eyring equation (*23*)

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

the enthalpy of activation being $\Delta H^\# = 78 \pm 7$ kJ mol^{-1} (the negative sign of the entropy of activation, $\Delta S^\# = -70 \pm 12$ J K^{-1} mol^{-1}, is consistent with the increase of order accompanying the formation of the sorbate−NBP adduct).

The $\Delta H^\#$ values determined for NPB alkylation by β-propiolactone and β-butyrolactone (*19*)—both possibly carcinogenic for humans (*24*, *25*)—were $\Delta H^\# = 41$ and 47 kJ mol^{-1}, respectively. The high $\Delta H^\#$ value for NBP alkylation by potassium sorbate shows that its reactivity as an alkylating agent is much lower than that of more effective alkylating agents such as carcinogenic lactones.

Despite the weak alkylating power of the potassium sorbate, the results obtained in this work allow us to conclude that attention should be paid when using this species as a preservative, seeking the optimal conditions for its use as well as when establishing suitable expiration times for products preserved with this chemical.

Absorption Coefficient of the Sorbate−NBP Adduct. We were also interested in knowing the molar absorption coefficient of the sorbate−NBP adduct. Knowledge of its value should permit easy determination of the concentration of the adduct by simply measuring the absorbance.

Several experiments were performed using $[S]_0 = 0.2$ M, $[HCl] = 0.1$ M, and six NBP concentrations in the $(1-6) \times 10^{-3}$ M range. When absorbance reached a plateau (see **Figures 2a** and **5a**), we assumed that the reaction of NBP with sorbate had reached 100%. **Figure 5** shows the results and confirms the good accuracy of the working method. The plot in **Figure 5b** shows the absorption coefficient to be $\epsilon = 204 \pm 17$ M^{-1} cm^{-1} ($\lambda = 580$ nm).

To rationalize the ϵ value measured in this work for the sorbate−NBP adduct, its structure was obtained by a geometry optimization. **Figure 6** shows the result.

The structure of the sorbate−NBP adduct reveals the existence of a lack of coplanarity of the two NBP-phenyl rings with a folding angle of ∼119°. Disruption of the π-electron cloud to interlink the two phenyl rings would lead to a small ϵ value, as was observed.

From the present study, the following conclusions arise:

(i) Potassium sorbate shows alkylating capacity on the nucleophile NBP, 4-(*p*-nitrobenzyl)pyridine, a trap for alkylating agents with nucleophilic characteristics similar to those of DNA bases.

(ii) The NBP alkylation reaction complies with the rate equation $r = k_{alk}[H^+][S][NBP]/(K_a + [H^+])$, K_a being the sorbic acid dissociation constant and k_{alk} the NBP alkylation rate constant by the undissociated acid. In the pH 5−6 range, the alkylation time ranges between 18 days (pH 5.2) and >1 month (pH ≥6).

(iii) NBP alkylation occurs through a reaction with $\Delta H^\# = 78$ kJ mol^{-1}, which is much higher than those of NBP alkylation reactions by stronger alkylating agents such as carcinogenic lactones.

(iv) The absorption coefficient of the sorbate−NBP adduct was determined to be $\epsilon = 204$ M^{-1} cm^{-1} ($\lambda = 580$ nm), this small value being due to the disruption of the π-electron cloud interlinking the two phenyl rings in the adduct.

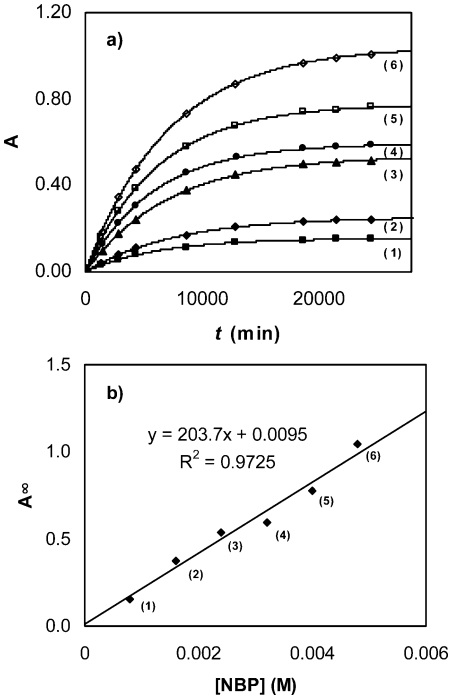


Figure 5. Absorption coefficient of the sorbate–NBP adduct in 7:3 water/dioxane medium: (**a**) variation in absorbance with time, A_∞ being the absorbance of the adduct when all of the NBP has been consumed; (**b**) determination of the mean absorption coefficient value. $[S]_0 = 0.2$ M; [NBP] $= 0.8 \times 10^{-3}$ M (1), 1.6×10^{-3} M (2), 2.4×10^{-3} M (3), 3.2×10^{-3} M (4), 4.0×10^{-3} M (5), and 4.8×10^{-3} M (6); [HCl] $= 0.1$ M; $\lambda = 580$ nm; $T = 35$ °C.

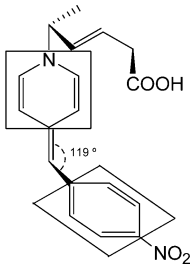


Figure 6. Lack of coplanarity in the sorbate–NBP adduct.

(v) The results can help to establish suitable expiration times for products preserved with potassium sorbate.

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