International Journal of Pharmaceutics, 66 (1990) 149-155
Elsevier

IJP 02259

The nucleophilicity of dextrose, sucrose, sorbitol, and mannitol with *p*-nitrophenyl esters in aqueous solution

Robert B. Killion, Jr* and Valentino J. Stella
Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS 66045 (U.S.A.)

(Received 4 June 1990)
(Modified version received 30 July 1990)
(Accepted 31 July 1990)

Key words: Dextrose; Sucrose; Sorbitol; Mannitol; *p*-Nitrophenyl ester; Nucleophilicity

Summary

Dextrose, sucrose, sorbitol, and mannitol accelerate the hydrolysis of simple *p*-nitrophenyl esters in aqueous solution. These compounds are polyhydric alcohols with pK_a values in the range of 12 to 13, and were found to be nucleophilic in the reaction with simple *p*-nitrophenyl esters in aqueous solutions buffered at neutral or slightly alkaline pH. The second-order rate constants for the nucleophilic reaction of dextrose, sucrose, sorbitol, and mannitol with *p*-nitrophenyl acetate were determined at 25°C and ionic strength 1.0 (KCl). Based on the pH dependency of the catalytic rate constants, the reactivity of the polyhydric alcohols was attributed to the anion derived from ionization of a hydroxy group in the polyhydric alcohol. The second-order rate constants (k_{RO^-}) representing the nucleophilic reactivity of the polyhydric alcohol anions were determined. A Brønsted plot of log k_{RO} vs pK_a of the polyhydric alcohols showed slight downward deviations when compared to the analogous correlation with alcohols and phenols of lower pK_a. The downward deviations increased as the pK_a increased. Similar deviations have been previously observed for other alcohols with $pK_a \geqslant 12$, and the deviations have been attributed to an exceptionally strong solvational energy barrier of these highly basic anions. Therefore, it appears that the nucleophilic reactivity of dextrose, sucrose, sorbitol and mannitol is similar to other alcohols of comparable pK_a. The nucleophilic reactivity of dextrose ($pK_a = 12.38$) with a series of alkyl substituted *p*-nitrophenyl esters was studied. The resulting Hammett correlation, log k_{RO} vs σ_I, gave $\rho_I = 6.12 \pm 0.10$. Similar ρ_I values have been reported for the reaction of other alkoxides with *p*-nitrophenyl esters.

Introduction

Numerous studies have shown that dextrose catalyzes the hydrolysis of β-lactam antibiotics in intravenous solutions (e.g. 5% dextrose in water, D5W) at neutral or alkaline pH values (Lynn et al., 1972; Landersjo et al., 1974, 1977, Bundgaard et al., 1978, 1979, 1983; Larsen et al., 1978; Stjernstrom et al., 1978; Stella 1986). Other carbohydrates including glucose, fructose, sucrose and dextrans, and polyhydric alcohols such as sorbitol, mannitol and glycerol have also been shown to be catalytically active in the hydrolysis of cephalosporins in aqueous solution (Bundgaard et al., 1983). The rate-accelerating effect of the polyhydric compounds was attributed to a nucleophilic reaction mechanism involving opening of the β-lactam moiety by an alkoxide ion derived from proton ionization of one of the hydroxyl groups generating an intermediate ester, which undergoes further hydrolysis. Since the pK_a values of most carbohydrates and polyhydric alcohols are

---

Correspondence: V.J. Stella, Dept. of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS 66045, U.S.A.
**Present address:* Syntex Research, Palo Alto, CA, U.S.A.

approximately 12 to 13, it is surprising that the reaction mechanism is attributed to the nucleophilic reaction of the *anion*. In fact, the concentration of the anion is extremely small at pH 7 for an alcohol with pK_a of 12 to 13. However, it must be realized that an alkoxide is a strong nucleophile, and is capable of nucleophilic reactions even when present at very low concentrations.

In order to better understand the nucleophilicity of carbohydrates and polyhydric alcohols, the reactivities of dextrose, sucrose, sorbitol, and mannitol with a few simple *p*-nitrophenyl esters were studied. The reactivity of many nucleophiles, including alkoxides and phenoxides with *p*-nitrophenyl acetate has been well documented (Jencks et al., 1982). Therefore, the reactivities of dextrose, sucrose, sorbitol, and mannitol to the reactivity of other alkoxide nucleophiles, according to a linear free energy relationship, were compared. To our knowledge, the reactivity of dextrose, sucrose, sorbitol, and mannitol with simple esters has not been reported. It should be noted that these materials are frequently used in various pharmaceutical formulations (Osol et al., 1980).

Experimental

The kinetics were monitored spectrophotometrically using a Cary 118 spectrophotometer with the cell compartment equilibrated at 25°C using a Forma Scientific 2095 circulating water bath.

Dextrose (Fisher), sucrose (Fisher), sorbitol (Aldrich), mannitol (Fisher), and KCl (Aldrich) were reagent grade chemicals and were used without further purification. The water used was deionized and glass distilled (Mega-Pure system model MP-1, Corning).

Solutions. Dextrose, sucrose, sorbitol, or mannitol was dissolved at various concentrations in either 0.10 M Tris or 0.05 M phosphate aqueous buffer solutions at various pH values, and the solutions were maintained at a constant ionic strength of 1.0 with KCl and buffer concentration. A 0.05 M stock solution of the ester substrate was prepared in acetonitrile.

Kinetics. The reactions of dextrose, sucrose, sorbitol, or mannitol (ROH) with *p*-nitrophenyl esters were conducted under pseudo first-order conditions with [ROH] >> [ester]. The kinetics of reaction were monitored spectrophotometrically at 351 nm, representing the appearance of *p*-nitrophenol. The temperature was 25°C, and the ionic strength was maintained at 1.0 with KCl.

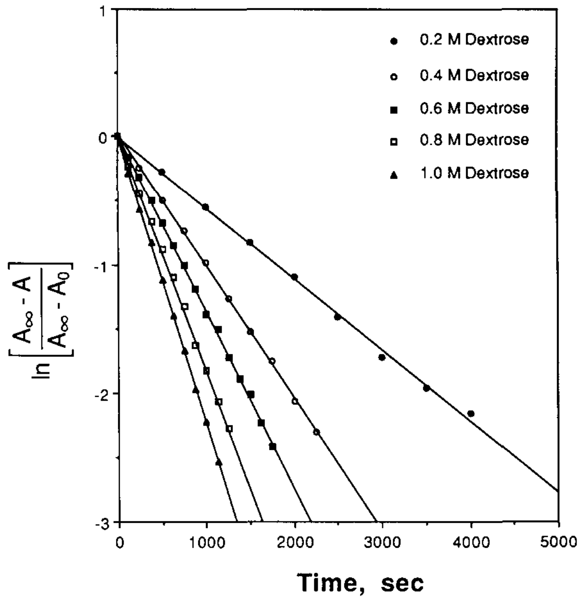


Fig. 1 Reaction of dextrose with *p*-nitrophenyl acetate at pH 8.32, at 25°C, ionic strength 1.0 (KCl). Kinetics of reaction monitored spectrophotometrically at 351 nm. The kinetics were run under pseudo first-order conditions with [*p*-nitrophenyl acetate]$_0$ of 8.33×10^{-5} M.

Typically, 3 ml of a buffered dextrose solution was equilibrated at 25°C for 10 min. The reactions were initiated by adding 5µl of a 0.05 M stock solution of the ester, and the kinetics were monitored continuously at 351 nm. Plots of ln $(A_\infty - A)/(A_\infty - A_0)$ vs time were linear for at least three half-lives, where A is the absorbance at time t, A_0 is the absorbance at time zero, and A_∞ is the absorbance at infinity (Moore et al., 1982). Representative plots are shown in Fig. 1 for the reaction of dextrose with *p*-nitrophenyl acetate at pH 8.32.

Results

The reaction of a polyhydric alcohol (ROH) and polyhydric alcohol anion (RO^-) with a typical *p*-

nitrophenyl ester (**S**) is shown in Scheme 1.

The rate law for the reaction shown in Scheme 1 is defined by Eqn 1, where k_{ROH} is the second-order rate constant representing the nucleophilic reaction of the neutral polyhydric alcohol (ROH) with the ester, k_{RO^-} is the second-order rate constant representing the nucleophilic reaction of the ionized polyhydric alcohol (RO^-) with the ester, and k_{hyd} is the spontaneous hydrolytic rate constant in the absence of the polyhydric alcohol.

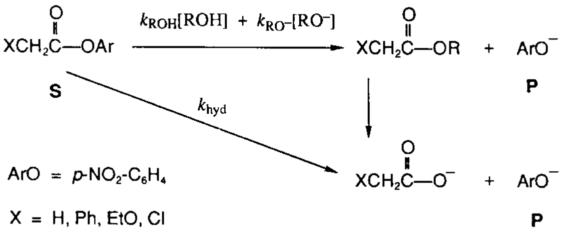
$$-\frac{\mathrm{d[S]}}{\mathrm{d}t}=k_{\mathrm{RO}^-}[\mathrm{RO}^-][\mathrm{S}]+k_{\mathrm{ROH}}[\mathrm{ROH}][\mathrm{S}]+k_{\mathrm{hyd}}[\mathrm{S}] \quad (1)$$

In principle, k_{hyd} is a function of pH and buffer concentration, however, these effects were not evaluated in our experiments. However, k_{hyd} was determined independently by studying the hydrolysis in the absence of added polyhydric alcohol. Using dextrose as an example, the concentration of dextrose anion, $[\mathrm{RO}^-]$, and the concentration of dextrose, [ROH], is related to the total concentration of dextrose $[\mathrm{ROH}]_{\mathrm{total}}$ according to Eqns 2 and 3, where K_a is the acid dissociation constant of dextrose.

$$[\mathrm{RO}^-]=\frac{K_a}{K_a+a_{\mathrm{H}^+}}[\mathrm{ROH}]_{\mathrm{total}}\approx\frac{K_a}{a_{\mathrm{H}^+}}[\mathrm{ROH}]_{\mathrm{total}} \quad (2)$$

$$[\mathrm{ROH}]=\frac{a_{\mathrm{H}^+}}{K_a+a_{\mathrm{H}^+}}[\mathrm{ROH}]_{\mathrm{total}}\approx[\mathrm{ROH}]_{\mathrm{total}} \quad (3)$$

The approximations are valid when $\mathrm{pH} << \mathrm{p}K_a$ ($a_{\mathrm{H}^+} >> K_a$). Eqn 1 simplifies to Eqn 4 under pseudo first-order conditions, i.e., $[\mathrm{ROH}]_{\mathrm{total}} >> [\mathrm{S}]$ with k_{obs} defined by Eqn 5.



Scheme 1.

$$-\frac{\mathrm{d[S]}}{\mathrm{d}t}=k_{\mathrm{obs}}[\mathrm{S}]=\frac{\mathrm{d[P]}}{\mathrm{d}t} \quad (4)$$

$$k_{\mathrm{obs}}=\left(\frac{K_a}{a_{\mathrm{H}^+}}k_{\mathrm{RO}^-}+k_{\mathrm{ROH}}\right)[\mathrm{ROH}]_{\mathrm{total}}+k_{\mathrm{hyd}} \quad (5)$$

The kinetics of substrate disappearance (−d[S]/dt) may be conveniently monitored spectrophotometrically by measuring the rate of p-nitrophenol appearance (d[**P**]/dt).

Experimentally, k_{obs} was determined as a function of total dextrose concentration at constant pH, buffer concentration, and ionic strength. Plots of k_{obs} vs $[\mathrm{ROH}]_{\mathrm{total}}$ for the reaction of dextrose with p-nitrophenyl acetate are shown in Fig. 2 at various pH values according to Eqn 5. Intercept values corresponded well with k_{hyd} determined independently in the absence of dextrose. Fig. 3 shows a plot of the slopes generated from

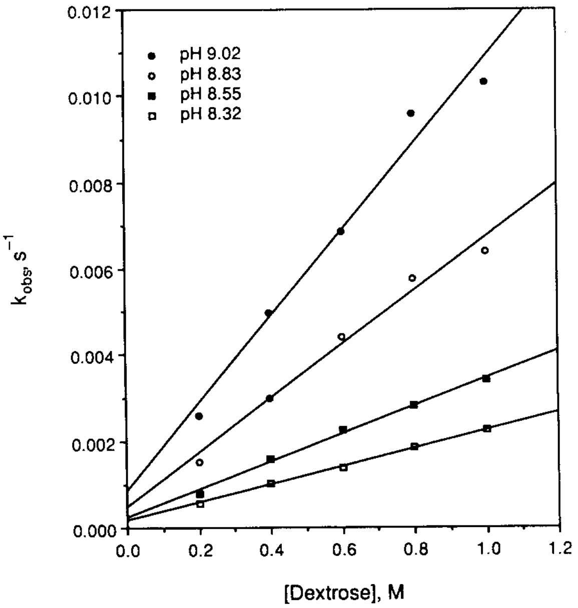


Fig. 2 Reaction of dextrose with p-nitrophenyl acetate at 25°C and ionic strength 1.0 (KCl). Plot of k_{obs} vs concentration of dextrose at various pH values according to Eqn 5.

linear fits to Eqn 5 vs $1/a_{H^+}$ for the reaction of dextrose with *p*-nitrophenyl acetate. The intercept of Fig. 3 is indistinguishable from zero, indicating that the relative contribution to k_{obs} coming from the nucleophilic reaction of dextrose (k_{ROH}) is negligible compared to the contribution coming from the nucleophilicity of dextrose anion (k_{RO^-}). Since K_a for dextrose is known (Wolley et al., 1982), the value of the second-order rate constant (k_{RO^-}) for the nucleophilicity of dextrose anion may be determined from the slope ($K_a k_{RO^-}$) of Fig. 3.

Kinetically equivalent terms to $k_{RO^-}\{RO^-][S]$ such as $k_{ROH}[ROH][S]a_{^-OH}$ cannot be distinguished from the present data. However, Bundgaard et al. (1978, 1979, 1983) and Jencks et al. (1982) assume the kinetic scheme proposed here, i.e., the catalytic effect of phenols, alcohols and polyhydric alcohols in ester and amide hydrolysis involves nucleophilic attack by the alcohol anion on the ester or amide rather than a mechanism involving the alcohol facilitating hydroxide attack on the ester or a concerted hydroxide ion abstraction of a proton during nucleophilic attack by the alcohol on the ester.

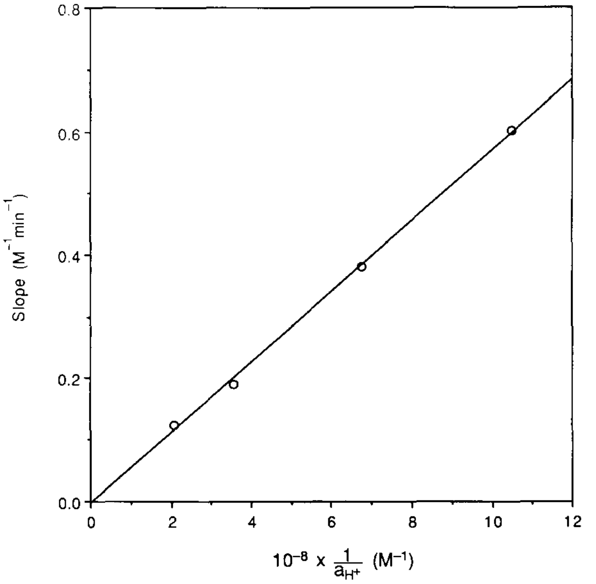
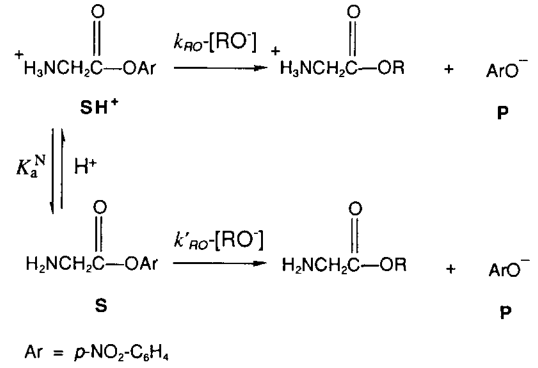


Fig. 3 Plot of the slope of data fit to Eqn 5 vs $1/a_{H^+}$ for the reaction of dextrose with *p*-nitrophenyl acetate.



Scheme 2.

The reaction of *p*-nitrophenyl glycinate is slightly more complicated since the neutral ester (**S**) is in equilibrium with its protonated form (**SH**$^+$), as shown in Scheme 2.

The rate constants corresponding to the reaction of the two glycinate species in the absence of the polyhydric alcohols were excluded from Scheme 2 for clarity. In the derivation of the rate law for the reaction shown in Scheme 2, the contribution to k_{obs} resulting from the nucleophilicity of dextrose (k_{ROH}) was considered to be negligible compared to the nucleophilicity of dextrose anion (k_{RO^-}) as observed previously for the other ester substrates. The rate law for the reaction shown in Scheme 2 is defined by Eqn 6, where k_{RO^-} and k'_{RO^-} are the second-order rate constants for the reaction of dextrose anion with **SH**$^+$ and **S**, respectively.

$$-\frac{d[S]}{dt} = k_{RO^-}[RO^-][S] + k'_{RO^-}[RO^-][SH^+] + k_{hyd}[S]_{total} \qquad (6)$$

where

$$[S]_{total} = [SH^+] + [S]$$

and

$$K_a^N = \frac{[S]\, a_{H^+}}{[SH^+]}$$

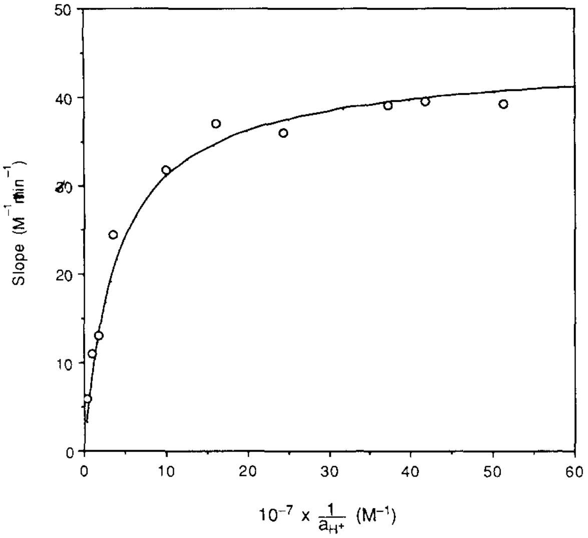


Fig. 4 Plot of the slope according to Eqn 7 vs $1/a_{H^+}$ for the reaction of dextrose with *p*-nitrophenyl glycinate.

Eqn 6 may be simplified under pseudo first-order conditions, i.e., $[ROH]_{total} >> [S]$, with k_{obs} defined according to Eqn 7.

$$k_{obs} = \left\{\frac{a_{H^+}k_{RO^-} + K_a^N k'_{RO^-}}{K_a^N + a_{H^+}}\right\} \times \frac{K_a}{a_{H^+}}[ROH]_{total} + k_{hyd} \qquad (7)$$

Plots of k_{obs} vs $[ROH]_{total}$ were linear (data not shown). Fig. 4 shows that a plot of the slope according to Eqn 7 vs $1/a_{H^+}$ is curvilinear with an intercept of zero upon extrapolation of $1/a_{H^+}$ to zero. The solid line in Fig. 4 was generated assuming values of k_{RO^-} of 2.6×10^6 M^{-1} min^{-1}, k'_{RO^-} of 3×10^3 M^{-1} min^{-1}, and a pK_a^N value of 7.6.

Brønsted plot

Table 1 summarizes the second-order rate constants k_{RO^-} and pK_a values for the reaction of dextrose, sucrose, sorbitol, and mannitol. In order to assess the catalytic activity of these nucleophiles, a Brønsted plot of log k_{RO^-} vs pK_a was made for a series of oxyanion nucleophiles, as shown in Fig. 5. The solid line represents the Brønsted equation

TABLE 1

Summary of second-order rate constants k_{RO^-} and pK_a values for the reaction of dextrose, sucrose, mannitol, and sorbitol with p-nitrophenyl acetate at 25°C and ionic strength 1.0 (KCl)

Polyhydric alcohol	pK_a[a]	k_{RO^-}(±SD) (M^{-1} min^{-1})
Dextrose	12.38	$1.45(\pm 0.07) \times 10^3$
Sucrose	12.75	$1.26(\pm 0.06) \times 10^3$
Mannitol	13.47	$2.24(\pm 0.11) \times 10^3$
Sorbitol	13.60	$2.78(\pm 0.14) \times 10^3$

[a] Wolley et al. (1972); Merck Index, 10th Edn, p. 818 (mannitol) and p. 1248 (sorbitol).

$$\log k_{RO^-} = 0.71 pK_a - 5.08 \qquad (8)$$

The data points for phenoxides and alkoxides were taken from the literature (Jencks et al., 1982). The simple alkoxides and polyhydric alkoxides ($pK_a > 12$) deviate downward from the linear Brønsted correlation observed for phenoxides ($pK_a < 12$). This phenomenon has been previously described for strongly basic alkoxides ($pK_a > 12$), and was attributed to an exceptionally strong solvational energy barrier (Hupe et al., 1977; Jencks et al., 1982). The data points for the alkoxides are

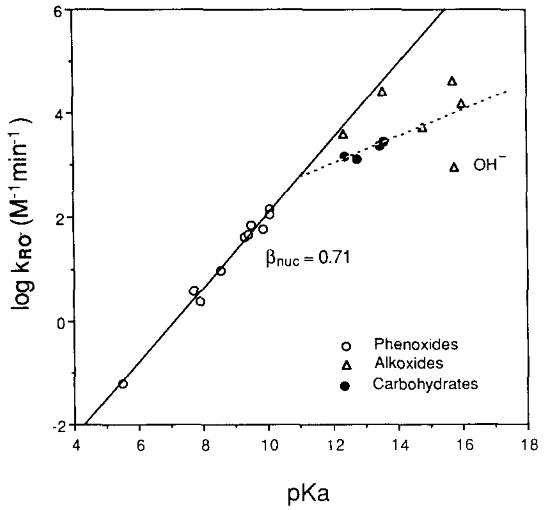


Fig. 5 Brønsted plot of log k_{RO^-} (M^{-1} min^{-1}) vs pK_a at 25°C and ionic strength 1.0 (KCl) for the nucleophilic reaction of phenoxides, alkoxides, and carbohydrates with *p*-nitrophenyl acetate. The solid line represents the Brønsted equation log k_{RO^-} = 0.71 pK_a − 5.08. The dashed line drawn through the points for the carbohydrates has a slope of 0.25.

TABLE 2

Summary of second-order rate constants k_{RO^-} and σ_I values for the reaction of dextrose with various alkyl substituted p-nitrophenyl esters at 25°C and ionic strength 1.0 (KCl)

Ester	σ_I[a]	k_{RO^-}(±SD) ($M^{-1}\,min^{-1}$)
CH_3COOAr	0	$1.45(\pm 0.07)\times 10^3$
$PhCH_2COOAr$	0.12	$4.79(\pm 0.24)\times 10^3$
H_2NCH_2COOAr	0.12	$3.00(\pm 0.15)\times 10^3$
$EtOCH_2COOAr$	0.29	$3.72(\pm 0.19)\times 10^4$
$ClCH_2COOAr$	0.47	$5.71(\pm 0.29)\times 10^5$
$CH_3(NH_3^+)CHCOOAr$	0.55	$4.93(\pm 0.25)\times 10^6$
$^+H_3NCH_2COOAr$	0.60	$2.60(\pm 0.13)\times 10^6$

Ar = p-NO_2-C_6H_4.
[a]Hammett (1970).

somewhat scattered, but in general show similar downward deviations from the Brønsted line. The dashed line in Fig. 5 has a slope of 0.25, and fits the data for the polyhydric alcohols.

Hammett Plot

The reactivity of dextrose anion with various substituted p-nitrophenyl esters depends on the ester substituent. Table 2 summarizes the second-order rate constants k_{RO^-} and σ_I values (Hammett, 1970). Fig. 6 shows a Hammett plot of log k_{RO^-} vs σ_I (Hammett, 1970). A linear correlation was observed, and the data fits the Hammett equation

$$\log k_{RO^-} = 6.12\ (\pm 0.10)\sigma_I + 2.94 \qquad (9)$$

The value of $\rho_I = 6.12 \pm 0.1$ is characteristic of an alkoxide nucleophile (Holmquist et al., 1969), and is consistent with the nucleophilic reactivity of dextrose anion.

Conclusion

The polyhydric alcohols dextrose, sucrose, sorbitol, and mannitol react with simple p-nitrophenyl esters by an apparent nucleophilic mechanism in neutral to alkaline aqueous solution. The kinetic parameters for the reaction of these polyhydric alcohols were evaluated. It was found that the reactivity can be attributed to the alkoxide derived from ionization of a hydroxyl group (Haines, 1976) in the polyhydric alcohol. The log k_{RO^-} values for the nucleophilic reaction of the polyhydric alcohols with p-nitrophenyl acetate were correlated with the pK_a of the alcohol. The resulting Brønsted plot showed that the log k_{RO^-} values for the polyhydric alcohols deviated slightly below the line for the correlation of phenoxides. The deviations became stronger as the pK_a of the alcohol increased. A similar phenomenon has been reported for alkoxides with pK_a > 12, and was attributed to an additional energy barrier resulting from strong solvation of the alkoxide (Hupe et al., 1977; Jencks et al., 1982).

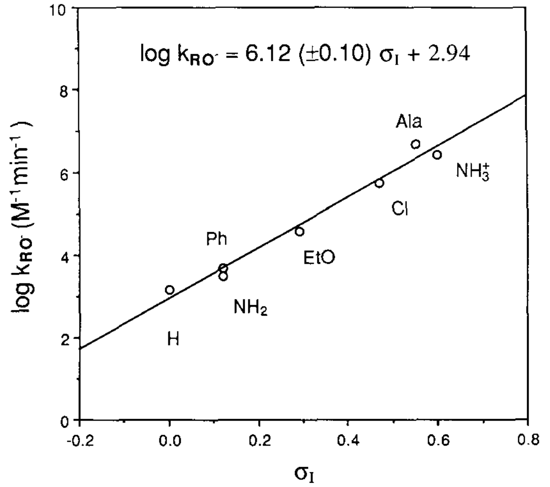


Fig. 6 Hammett plot of log k_{RO^-} ($M^{-1}\,min^{-1}$) representing second-order rate constants for the nucleophilic reaction of dextrose anion with various substituted esters $XCH_2COOC_6H_4$-p-NO_2 (X = H, Ph, EtO, Cl, H_2N, and H_3N^+), vs σ_I at 25°C and ionic strength 1.0 (KCl).

The reactivity of dextrose with various substituted p-nitrophenyl esters was studied by variation of the alkyl substituent on the ester substrate. The log k_{RO^-} values for the reaction of dextrose with the various substituted p-nitrophenyl esters was correlated with σ_I. The resulting Hammett plot ($\rho_I = 6.12 \pm 0.10$) is characteristic of alkoxide nucleophiles, and is consistent with the reactivity of dextrose anion resulting from ionization of a hydroxy group with pK_a of 12.38.

It can be concluded that the polyhydric alcohols, dextrose, sucrose, sorbitol, and mannitol display nucleophilic reactivity with simple activated esters in aqueous solution buffered at neutral to alkaline pH, and that the nucleophilic reactivity may be attributed to the anion resulting from ionization of a hydroxy group. The data supports the previously published observations that dextrose and other carbohydrates react with activated amides such as the β-lactam antibiotics in intravenous solutions buffered at neutral to alkaline pH values. These results should be considered when a drug that contains an ester or amide functional group, especially an activated ester or amide, is formulated in an aqueous solvent that contains a polyhydric alcohol as an additive.

Acknowledgements

This work was supported by the National Cancer Institute, Contracts NO1-CM-67912 and NO1-CM-97576

References

Bundgaard, H. and Larsen, C., Kinetics and mechanism of the sucrose-accelerated degradation of penicillins in aqueous solution. *Int. J. Pharm.*, 1 (1978) 95–104.

Bundgaard, H. and Larsen, C., The influence of carbohydrates and polyhydric alcohols on the stability of cephalosporins in aqueous solution. *Int. J. Pharm.*, 16 (1983) 319–325.

Bundgaard, H. and Larsen, C., Piperazinedione formation from reaction of ampicillin with carbohydrates and alcohols in aqueous solution. *Int. J. Pharm.*, 3 (1979) 1–11.

Hammett, L., In *Physical Organic Chemistry* 2nd Edn, McGraw-Hill, New York, 1970.

Haines, A.H., Relative reactivities of hydroxyl groups. *Adv. Carbohyd. Chem. Biochem.*, 33 (1976) 11–109.

Holmquist, B. and Bruice, T.C., Electrostatic catalysis. III. Comparison of the reactivity of α-substituted *o*-nitrophenyl esters with anionic and amine nucleophiles. *J. Am. Chem. Soc.*, 91 (1969) 2985–2993.

Hupe, D.J. and Jencks, W.P., Nonlinear structure-reactivity correlations. Acyl transfer between sulfur and oxygen nucleophiles. *J. Am. Chem. Soc.*, 99 (1977) 451–464.

Jencks, W.P., Brant, S.R., Gandler, J.R., Fencrich, G. and Nakamura, C, Nonlinear Brønsted correlations: The role of resonance, solvation, and changing transition-state structure. *J. Am. Chem. Soc.*, 104 (1982) 7045–7051.

Landersjo, L., Kallstrand, G. and Lundgren, P., Studies on the stability and compatibility of drugs in infusion fluids. III. Factors affecting the stability of cloxacillin. *Acta Pharm. Suecica,* 11 (1974) 563–580.

Landersjo, L., Stjernstrom, G. and Lundgren, P., Studies on the stability and compatibility of drugs in infusion fluids. IV. Factors affecting the stability of benzylpenicillin in carbohydrate solutions. *Acta Pharm. Suecica,* 14 (1977) 293–308.

Larsen, C. and Bundgaard, H., Penicilloyl ester intermediates in glucose· and fructose-accelerated degradation of benzylpenicillin in aqueous solution. *Arch. Pharm. Chem. Sci. Ed.*, 6 (1978) 3340.

Lynn, B., Stability of methicillin in dextrose solutions at alkaline pH. *J. Hosp. Pharm.*, 30 (1972) 81–83

Moore, J.W. and Pearson, R.G., *Kinetics and Mechanism*, Wiley, New York, 1981, Ch. 2.

Osol, A., Chase, G.D., Gennaro, A.R., Gibson, M.R., Granberg, C.B., Harvey, S.C., King, R.E., Martin, A.N., Swinyard, E.A. and Zink, G.L., *Remington's Pharmaceutical Sciences*, Mack, Easton, PA, 1980.

Stella, V.J., Chemical and physical bases determining the instability and incompatibility of formulated injectable drugs. *J. Parent. Sci. Technol.,* 40 (1986) 142–163.

Stjernstrom, G., Olson, O.T., Nyqvist, H. and Lundgren, P, Studies on the stability and compatibility of drugs in infusion fluids. IV. Factors affecting the stability of ampicillin. *Acta Pharm. Suecica,* 15 (1978) 33–50.

Wolley, E.M., Tomkins, J. and Hepler, L.G., Ionization constants for very weak organic acids in aqueous solution and apparent ionization constants for water in aqueous organic mixtures. *J. Solution Chem.*, 1 (1972) 341–351.