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KINETICS AND MECHANISM OF THE SUCROSE-ACCELERATED DEGRADATION OF PENICILLINS IN AQUEOUS SOLUTION

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SUMMARY

The kinetics and mechanism of the degradation of benzylpenicillin and a number of semi-synthetic penicillins in aqueous sucrose solutions of pH 6–10 have been investigated at 35°C. A linear relationship between the degradation rate and the sucrose concentration up to 10% w/v was observed and the rate-accelerating effect of sucrose was found to be directly proportional to the hydroxide ion concentration in the investigated pH range.

The presence of a penicilloate ester of sucrose in the reaction pathway was demonstrated by penamaldate analysis and kinetic analysis showed that the sucrose-catalyzed hydrolysis of penicillins to penicilloic acids proceeds entirely through a nucleophilic pathway with an intermediate formation of penicilloyl sucrose esters.

INTRODUCTION

Reaction of penicillins with various carbohydrates in neutral and alkaline solutions has received increasing attention in recent years, both as regards stability and penicillin allergy. Penicillins are commonly infused in dextrose or fructose solutions and these carbohydrates have been shown to accelerate the degradation of penicillins at neutral and alkaline pH (Simberkoff et al., 1970; Lynn, 1972; Hem et al., 1973; Chatterji et al., 1975; Ashwin and Lynn, 1975; Landersjö et al., 1977). Sucrose, which is a common ingredient of penicillin syrups, has also been found to catalyze the inactivation of penicillins in neutral or alkaline solutions (Simberkoff et al., 1970; Hem et al., 1973).

The mechanism of these carbohydrate-accelerated degradations has not yet been established. Schneider and de Weck (1967) have reported that benzylpenicillin in aqueous solution at pH 7.4 is capable of penicilloylating hydroxyl groups in various carbohydrates including dextrose, fructose, sucrose, raffinose and higher molecular weight dextrans. Similarly, Tutt and Schwartz (1971) have demonstrated the formation of a penicilloate

ester intermediate in the cycloheptaamylose-catalyzed hydrolysis of penicillins in weakly alkaline solution. On the other hand, Hem et al. (1973) in their study of the effect of sucrose on penicillin stability have concluded that penicilloylation of hydroxyl groups does not seem to be involved as a significant degradation mechanism. Instead, these authors proposed the formation of a penicillin–sucrose complex, in which penicillin undergoes hydrolysis faster than the uncomplexed drug.

The penicilloate esters formed from reaction of benzylpenicillin with raffinose, inulin and various dextrans in neutral aqueous solution have been shown to be capable of eliciting penicilloyl-specific allergic reactions in sensitized animals (Schneider and de Weck, 1969; Schneider et al., 1971; Molinari et al., 1973), and therefore the presence of even small amounts of such penicilloyl–carbohydrate conjugates in clinically used penicillin preparations may play a part in the elicitation of penicillin allergic reactions.

The purpose of the present investigation was to study the kinetics of degradation of penicillins in aqueous sucrose solutions over a broad range of pH and sucrose concentration and to determine the mechanism by which the carbohydrate exerts its effect on penicillin degradation.

MATERIALS AND METHODS

Chemicals and apparatus

The penicillins studied (benzylpenicillin sodium, ampicillin sodium, carbenicillin disodium and phenoxymethylpenicillin potassium) were commercial products and were used as received. Sucrose was of pharmacopoeial quality. All of the other chemicals used were of reagent grade.

A Zeiss PMQ II spectrophotometer and 1-cm quartz cells were used for the ultraviolet spectral measurements. The pH measurements were made at the temperature of study using a Radiometer Type PHM 26 instrument equipped with a scale expander.

Analytical procedures

Assay for residual benzylpenicillin. This was done by the spectrophotometric method of Bundgaard and Ilver (1972).

Assay for penicilloic acid and penicilloyl ester. Determination of the concentrations of penicilloic acid and penicilloyl sucrose in the reaction solutions was performed by means of the spectrophotometric penamaldate assay of Schneider and de Weck (1966) modified by Schwartz and Delduce (1969). This assay (treatment of aliquot portions of the reaction solution at pH 7.0 (0.1 M phosphate buffer solution) with mercury (II) chloride and subsequent measurement of absorbance and absorbance-stability at 282 nm) is specific to penicilloic acids and penicilloyl derivatives (esters or amides), and permits distinction between them. The molar absorptivity of benzylpenicilloic acid (prepared by allowing a 2×10^{-3} M benzylpenicillin solution in 0.1 M sodium hydroxide to stand for 3 hr at room temperature) in the penamaldate assay was determined to be 9.40×10^3 dm^3 mol^{-1} cm^{-1}. For the calculation of the concentration of benzylpenicilloyl sucrose a molar absorptivity of 21.5×10^3 dm^3 mol^{-1} cm^{-1} was used. This value was the molar absorptivity of *N*-(benzylpenicilloyl)benzylamine, prepared as described by Levine (1962), in the penamaldate assay.

Kinetic measurements

All kinetic experiments were carried out in aqueous buffer solutions (citrate, borate or carbonate) at 35.0°C. The solutions containing varying amounts of sucrose were kept in a water bath in screw-capped test tubes. After addition of benzylpenicillin sodium to give an initial concentration of about 1.5×10^{-3} M, samples of 500 µl were taken at appropriate intervals and analyzed for undegraded penicillin, penicilloic acid and penicilloyl ester as described above. At constant pH the rate of disappearance of penicillin followed first-order kinetics. Pseudo-first-order rate constants were calculated from the slopes of linear plots of the logarithm of remaining penicillin against time.

The rates of degradation of a number of penicillins in 0.1 M carbonate buffer solution (pH 10.12 at 35°C) containing varying concentrations (2–10% w/v) of sucrose were determined by recording the decrease in absorbance at 236 nm. Cuvettes containing 2.5 ml of the sucrose solutions were thermally equilibrated in the thermostatted compartment of the spectrophotometer and the reactions were initiated by adding 25 µl of a freshly prepared aqueous solution of the penicillins to give a final concentration of about 10^{-3} M. Pseudo-first-order rate constants were calculated from plots of $\log(A_t - A_\infty)$ against time, where A_t is absorbance at 236 nm at time t and A_∞ absorbance at infinite time. This direct spectrophotometric assay has previously been used to measure rates of aminolysis and alkaline hydrolysis of the penicillins (Bundgaard, 1975 and 1976).

RESULTS AND DISCUSSION

Kinetics of sucrose-accelerated degradation of penicillins

The rates of degradation of benzylpenicillin in aqueous sucrose solutions were measured at 35°C over the pH range 6–10.1. With the molar sucrose concentration being in large excess over the penicillin concentration the loss of penicillin followed strict first-order kinetics at each pH. Values of the observed pseudo-first-order rate constants (k_{obs}) are listed in Table 1. In Figs. 1 and 2, the k_{obs} values are plotted versus sucrose concentration. The figures show that the rate of degradation of benzylpenicillin increases linearly with increasing sucrose concentration at each pH value and that the accelerating effect of sucrose on penicillin degradation increases with increasing pH. The results may be described by the following expression

$$k_{obs} = k_{hyd} + k_1 [\text{sucrose}] \quad (1)$$

where k_{hyd} represents the pseudo-first-order rate constants for hydrolysis of penicillin in absence of sucrose and k_1 is a pH-dependent apparent second-order rate constant for the reaction of penicillin with sucrose. When the logarithms of the slopes ($= k_1$) of the lines in Fig. 1 and of the line for benzylpenicillin in Fig. 2 were plotted against pH a straight line with a slope equal to 0.97 was obtained (Fig. 3). Thus, in the pH range 6–10.1 the sucrose reactions show a first-order dependence on hydroxide ion concentration. Eqn. 1 may accordingly be written as:

$$k_{obs} = k_{hyd} + k_1' [OH^-] [\text{sucrose}] \quad (2)$$

where k_1' has a value of 1.0×10^6 dm^6 mol^{-2} h^{-1} at 35°C.

The rate of degradation of other penicillins in aqueous solution was also found to be

TABLE 1

PSEUDO-FIRST-ORDER RATE CONSTANTS FOR DEGRADATION OF BENZYLPENICILLIN AT 35°C IN AQUEOUS SOLUTIONS WITH AND WITHOUT ADDITION OF SUCROSE

pH	Buffer	Sucrose conc. (%)	k_{obs} (h^{-1})
6.00	0.1 M citrate	0	3.6×10^{-3}
		2	4.0×10^{-3}
		5	4.3×10^{-3}
7.00	0.1 M citrate	0	1.8×10^{-3}
		2	4.3×10^{-3}
		5	7.7×10^{-3}
		10	14.5×10^{-3}
8.48	0.1 M borate	0	0.27×10^{-1}
		2	0.92×10^{-1}
		5	1.87×10^{-1}
		10	4.03×10^{-1}
9.10	0.1 M borate	0	0.74×10^{-1}
		2	0.31
		5	0.80
		10	1.55
10.12	0.1 M carbonate	0	0.78
		2	3.0
		5	6.8
		10	13.2

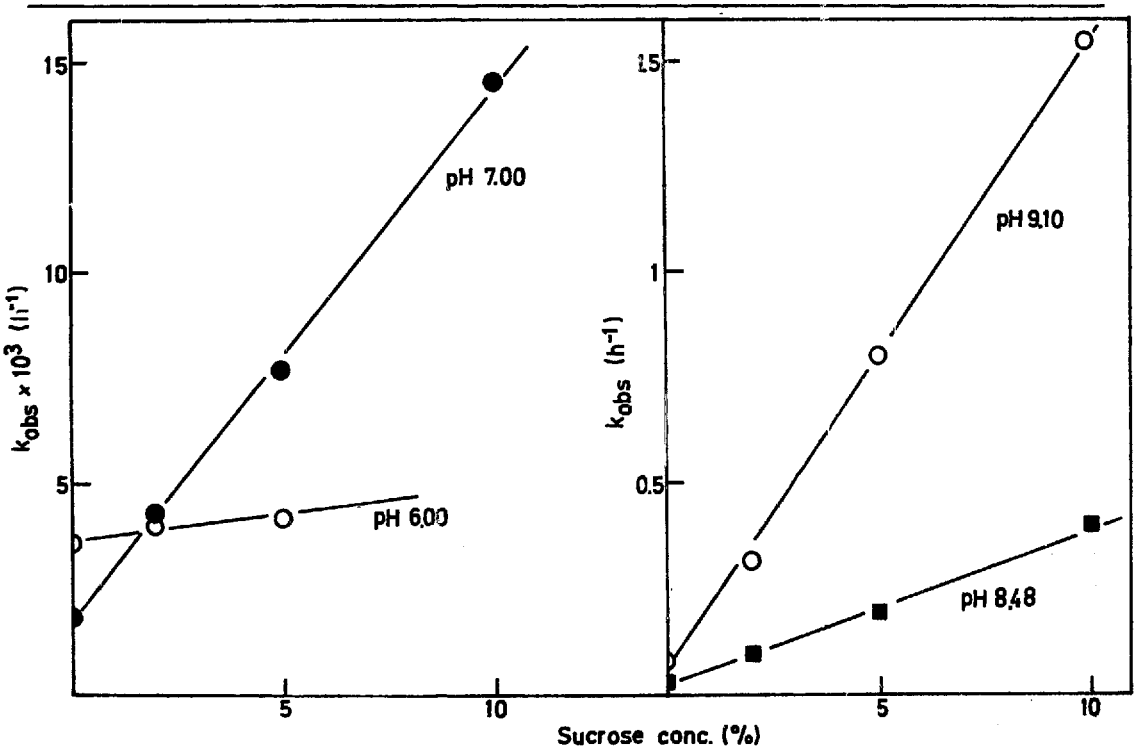
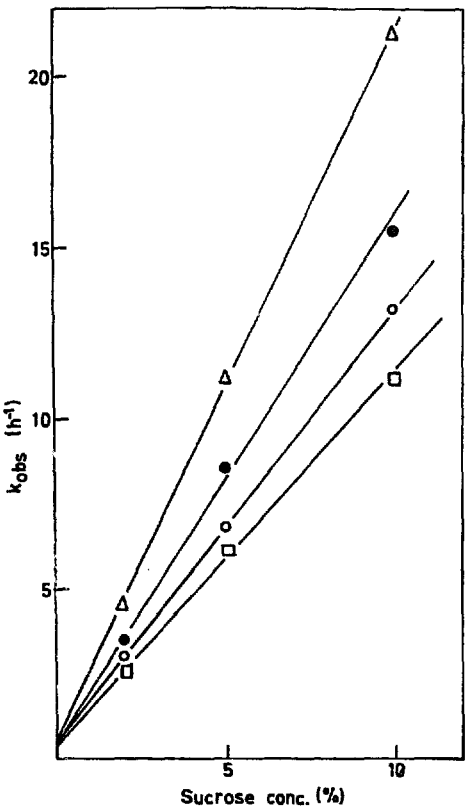


Fig. 1. Effect of sucrose on the pseudo-first-order rate constant for the degradation of benzylpencillin in aqueous solution at various pH values (35°C).



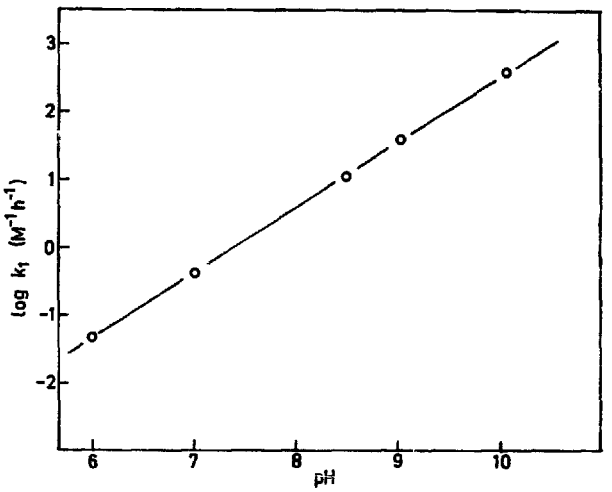


Fig. 2. Effect of sucrose on the pseudo-first-order rate constant for the degradation of various penicillins in 0.1 M carbonate buffer solution of pH 10.12 (35°C). Key: △, phenoxymethylpenicillin; •, ampicillin; ○, benzylpenicillin; □, carbenicillin.

Fig. 3. The logarithm of the apparent second-order rate constants for sucrose-accelerated degradation of benzylpenicillin plotted as a function of pH.

markedly enhanced by sucrose. As seen from Fig. 2 the pseudo-first-order rate constants for disappearance of ampicillin, carbenicillin, and phenoxymethylpenicillin in sucrose solutions at pH 10.12 increase strongly with the sucrose concentration, and also for these penicillins a linear dependence of k_{obs} on sucrose concentration was observed.

Although the penicillins differ only slightly in their reactivity with sucrose the variations as determined from the slopes of the lines in Fig. 2 were found to parallal the order of reactivity with hydroxide ions as expressed by the rate constants (k_{OH}) for hydroxide ion-catalyzed hydrolysis (Fig. 4). These constants were obtained from a previous study (Bundgaard, 1976).

Landersjö et al. (1977) have recently reported that small amounts of iron strongly accelerated the degradation of benzylpenicillin in fructose solutions at alkaline pH. The rate-accelerating effect of sucrose observed in the present study was not due to metal catalysis caused by trace contaminants nor was the sucrose reactions found to be catalyzed by iron ions in the presence of borate or carbonate buffers. Rate constants for the degradation of benzylpenicillin in 10% sucrose solution at pH 9.10 (0.1 M borate) and

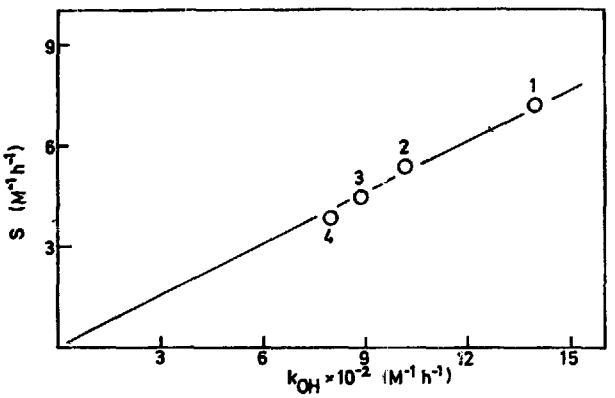


Fig. 4. Plot of the slopes (S) of the lines in Fig. 2 against the second-order rate constants for hydroxide ion-catalyzed hydrolysis of the corresponding penicillins. Key: 1, phenoxymethylpenicillin; 2, ampicillin; 3, benzylpenicillin; 4, carbenicillin.

10.12 (0.1 M carbonate) were unaffected by the addition of disodium edetate (10^{-3} M) or by the addition of iron (II) sulphate in amounts corresponding to 0.2 μg Fe^{2+} per ml.

The observed linear dependence of the apparent pseudo-first-order rate constants for degradation of the penicillins on the sucrose concentration does not agree with results reported by Hem et al. (1973). These authors studied the rate of degradation of a number of penicillins, including benzylpenicillin, as a function of sucrose concentration (up to 5%) at pH 7.0 (0.06 M citrate buffer) and 45°C and they described a non-linear dependence of k_{obs} on sucrose concentration. The reason for this discrepancy is unknown. At each pH studied we have not observed even a slight tendency of the plots of k_{obs} against sucrose concentration to level off although we have used sucrose concentration up to twice as high as those used by Hem et al. In this context it should be noted that the accelerating effect of fructose and dextrose on the rate of degradation of benzylpenicillin and oxacillin at pH 7–8 has also been shown to be linearly dependent on carbohydrate concentration (Landersjö et al., 1977; Chatterji et al., 1975).

Mechanism of sucrose reactions

The rate-accelerating effect of sucrose on the degradation of penicillins in neutral and alkaline solutions may be a result of either a general base catalysis of hydrolysis or of a nucleophilic reaction of an alkoxide ion derived from a hydroxyl group of the sucrose, or both. These reactions are kinetically equivalent, but they may be distinguished by product analysis since the nucleophilic pathway should involve a formation of penicilloylated sucrose with at least a transitory existence. A general base catalytic mechanism would result in the formation of only penicilloic acid.

Submitting aliquots of the reaction mixture of benzylpenicillin and sucrose to the penamaldate assay procedure at various intervals showed a rapid formation of a product behaving like a penicilloate ester and a slower formation of penicilloic acid. The penamaldate stability (i.e. the penamaldate absorbance value mueasured at 10 min from the time of addition of mercury (II) chloride in relation to the absorbance measured at 0 time (Schneider and de Weck, 1966)) of the product was 94–96%, which is characteristic of

penicilloyl derivatives (amides or esters). The corresponding value for penicilloic acid is 20–25%. This observation, along with the observation of a marked initial induction period in the formation of penicilloic acid (see Fig. 5), indicates the presence of a penicilloate ester of sucrose in the reaction pathway. The variations of benzylpenicillin, penicilloic acid and penicilloate ester concentrations, all determined experimentally, as a function of time at pH 8.48 are shown in Fig. 5. At any time the sum of the concentrations of the three species corresponded to 100 ± 5% in relation to the initial benzylpenicillin concentration.

The over-all reactions may be described by the following scheme:

$$\begin{array}{l} \text{BP + sucrose} \xrightarrow{k_{ef}} \text{ester} \xrightarrow{k_{ed}} \text{BPOH} \\ \downarrow k_{hyd} \\ \text{BPOH} \end{array}$$

where BP = benzylpenicillin and BPOH = benzylpenicilloic acid. The pseudo-first-order rate constants associated with the various reactions in this scheme were determined from the curves in Fig. 5 in the following manner. Treatment of the concentration–time data for benzylpenicillin and ester according to the procedure described by Niebergall and Sugita (1968) afforded the following values: $k_{ef} = 0.37\ h^{-1}$ and $k_{ed} = 0.17\ h^{-1}$. Since $k_{obs} = k_{hyd} + k_{ef}$ and $k_{obs} = 0.40\ h^{-1}$ (Table 1), $k_{hyd} = 0.03\ h^{-1}$. When k_{ed} was determined from a semi-logarithmic plot of the descending part of the curve representing hydrolysis of ester to penicilloic acid, a similar value ($0.12\ h^{-1}$) was obtained. An independent check of the value for the rate constant for ester formation (k_{ef}) was made by determining the *initial* rate of ester formation at the same experimental conditions (Fig.

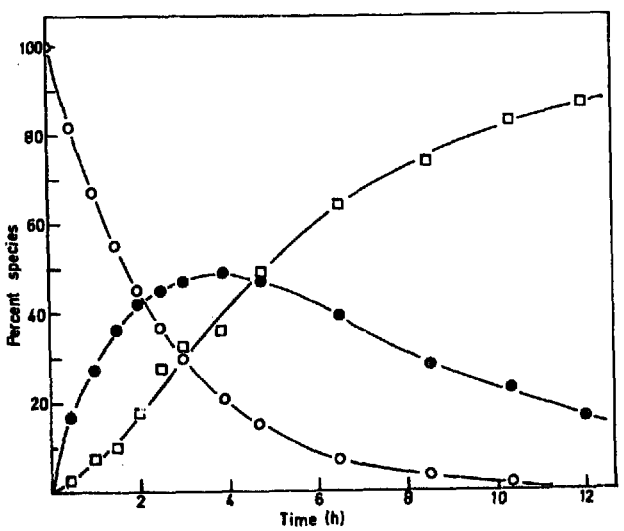


Fig. 5. Time-courses for benzylpenicillin (○), benzylpenicilloyl sucrose (●) and benzylpenicilloic acid (□) in the reaction between benzylpenicillin (1.4×10^{-3} M) and sucrose (10% w/v) at pH 8.48 (0.1 M borate) and 35°C. The concentrations at various times, expressed as percent in relation to the initial penicillin concentration, were determined by the imidazole method for benzylpenicillin and by the penamaldate assay for ester and penicilloic acid.

6). The slopes of the lines in Fig. 6 are related to k_{ef} by the equation

$$k_{ef} = \frac{\text{slope} \times N}{21.5 \times 10^3 \times [BP]_0} \quad (3)$$

where N represents the number of times by which an aliquot of the reaction mixture was diluted to give the penamaldate assay solution (= 11), the factor 21.5×10^3 is the assumed molar absorptivity of the penicilloyl sucrose in the assay and $[BP]_0$ is the initial benzylpenicillin concentration. The values for k_{ef} determined from such initial rate experiments at two sucrose concentrations were 0.34 and 0.35 h^{-1}, which are in close agreement with the value determined as described above.

It can be calculated from Table 1 that at pH 8.48 and at a sucrose concentration of 10% the rate effect of sucrose corresponds to a rate constant of $(k_{obs} - k_{hyd}) = 0.37\ h^{-1}$. Since the rate constant for ester formation has a value of 0.35–0.37 h^{-1} it can be concluded that the sucrose-catalyzed hydrolysis of penicillin proceeds largely or entirely through a nucleophilic pathway with an intermediate formation of a penicilloyl sucrose ester.

Similar results were obtained for reactions proceeding at pH 7.00. In solutions containing 10% sucrose the maximally occurring concentration of penicilloyl sucrose corresponded to 30 mol% of the initial penicillin concentration (Fig. 7). The following values were obtained for k_{ef} and k_{ed} at these conditions: $k_{ef} = 1.2 \times 10^{-2}\ h^{-1}$ and $k_{ed} = 1.7 \times 10^{-2}\ h^{-1}$. From a comparison of the k_{ed} values at pH 7.00 and 8.48 it appears that the hydrolysis of penicilloyl sucrose to penicilloic acid is subject to both hydroxide ion catalysis and water catalysis (spontaneous hydrolysis).

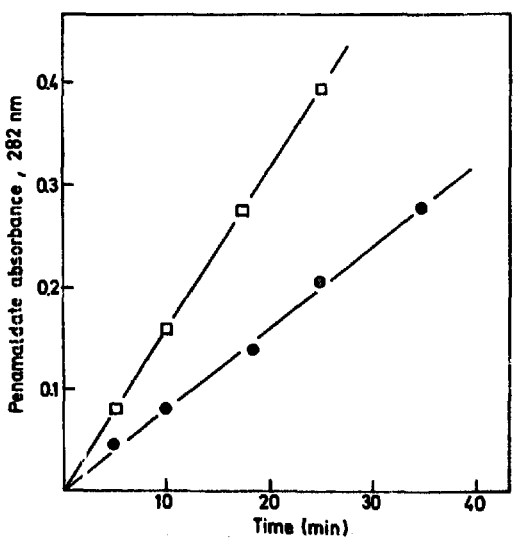


Fig. 6. The initial rate of formation of penicilloyl sucrose in aqueous solutions of pH 8.48 (0.1 M borate) and containing 5 (•) or 10% (□) of sucrose. The initial benzylpenicillin concentration was 1.4×10^{-3} M and aliquots of the reaction solutions were all diluted 11 times before being analyzed by the penamaldate assay.

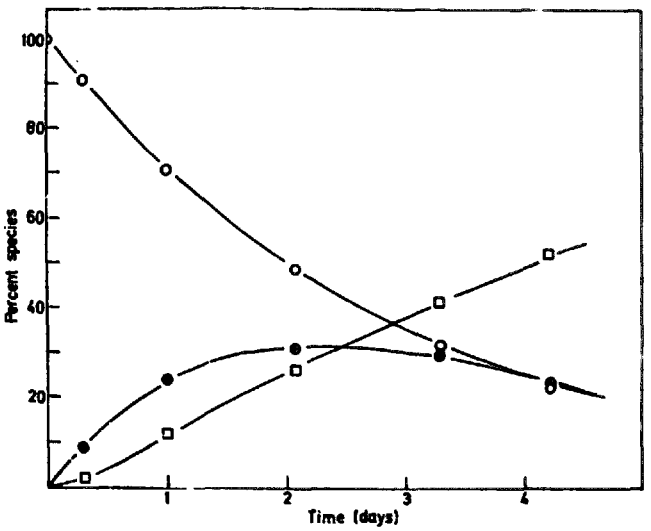


Fig. 7. Time-courses for benzylpenicillin (○), benzylpenicilloyl sucrose (●) and benzylpenicilloic acid (□) in the reaction between benzylpenicillin (1.4×10^{-3} M) and sucrose (10% w/v) at pH 7.00 (0.1 M citrate) and 35°C. The concentrations at various times, expressed as percent in relation to the initial penicillin concentration, were determined by the imidazole method for benzylpenicillin and by the penamaldate assay for ester and penicilloic acid.

The conclusion reached about the mechanism of the sucrose reactions differs from that proposed by Hem et al. (1973). In a study performed at pH 7 these authors found no indication of the formation of penicilloate esters. However, an application of specific methods, e.g. the penamaldate assay, to detect such esters has apparently not been made.

The observed first-order dependence of the sucrose reactions on the hydroxide ion concentration is consistent with the suggested mechanism. If, as proposed, the intermediate sucrose penicilloate is produced by a nucleophilic displacement reaction at the β-lactam carbonyl moiety by an alkoxide ion derived from one or more of the hydroxyl groups of the sucrose, it is reasonable to expect a direct proportionality between k_1 and hydroxide ion concentration within the pH range studied since this is much below the pK_a values for the sucrose hydroxyl groups. Reactions of penicillins with various phenols and trifluoroethanol in aqueous solutions have previously been shown to be due to a nucleophilic reaction mechanism involving formation of unstable penicilloate esters through displacement reactions by the corresponding oxygen anions (Bundgaard, 1976).

Penamaldate analysis of solutions of sucrose and the other penicillins studied also revealed a production of penicilloyl sucrose esters during the reaction progress.

In summary, it has been shown that the sucrose-accelerated degradation of penicillins in neutral and alkaline solutions is due to a nucleophilic displacement reaction with the formation of sucrose penicilloate esters. Since such compounds may be antigenic and capable of eliciting penicilloyl-specific allergic reaction in sensitized individuals (Molinari et al., 1973) their presence in penicillin preparations must be avoided. Keeping pH of solutions of penicillins and sucrose at 6–6.5 would be an efficient means to prevent the formation of sucrose penicilloate.

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