

Detection by Low Temperature Spectrophotometry of a Mixed Anhydride Intermediate in the Carboxylate Ion-catalysed Hydrolysis of a Sulphite Ester

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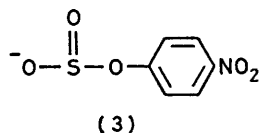
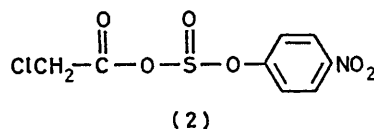
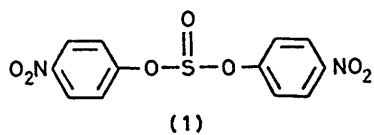
Summary By low temperature spectrophotometry in aqueous ethylene glycol solutions we have observed two phases corresponding to the release of *p*-nitrophenol in both stages of the chloroacetate ion-catalysed hydrolysis of bis(*p*-nitrophenyl) sulphite, and by trapping with hydroxylamine we have identified the intermediate formed in the first phase as the mixed anhydride.

THE carboxylate ion-catalysed hydrolysis of sulphite esters provides a useful model for the corresponding pepsin-catalysed reaction.¹ It has been shown² that acetohydroxamic acid is formed when the acetate ion-catalysed hydrolysis of diphenyl sulphite is carried out in the presence of hydroxylamine. This was interpreted as indicating that a mixed anhydride intermediate, resulting from attack of the acetate ion at the sulphite sulphur, had been trapped by the hydroxylamine. However, even by means of stopped-flow spectrophotometry, a technique which has detected intermediates in the decomposition of sulphite esters catalysed by other nucleophiles,² no direct spectrophotometric evidence for the mixed anhydride intermediate could be

adduced. In view of our successful use of low temperature spectrophotometry to establish the intermediacy of a mixed anhydride in the carboxypeptidase A-catalysed hydrolysis of an ester,³ we have examined whether a similar approach might be appropriate in the carboxylate ion-sulphite ester reaction.

We here report that by using low temperatures we have obtained spectrophotometric evidence for the intermediacy of a mixed anhydride in the chloroacetate ion-catalysed hydrolysis of bis(*p*-nitrophenyl) sulphite (**1**). Observation at -30.0°C of the hydrolysis of (**1**) in 50% ethylene glycol-50% aqueous solution containing chloroacetate (0.05—0.4 M, $\text{p}a_{\text{H}}^{\dagger}$ 3.05—3.7,⁴ $\mu = 0.4$ M) revealed a biphasic release of *p*-nitrophenol as monitored at 325 nm. Both phases of the reaction were found to follow pseudo-first order rate laws. The infinity absorbance calculated for the first, rapid phase of the reaction corresponded with the value expected for the release of one of the two incipient *p*-nitrophenol molecules which can be formed from bis(*p*-nitrophenyl) sulphite (**1**), suggesting the transient formation of an intermediate having either the mixed anhydride structure (**2**) or the monoester structure (**3**).

$\dagger \text{p}a_{\text{H}} \equiv \text{pH}$ apparent.

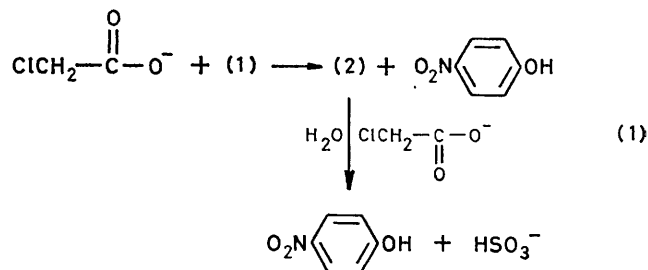


The observed rate constants, k_f , measured for the first phase of the reaction as a function of chloroacetate concentration, showed a linear dependence on the buffer concentration (0.05–0.4 M) at constant p_{aH} . At the chloroacetate concentrations employed, spontaneous hydrolysis of the sulphite ester could be neglected. Examination of the kinetics of the formation of the intermediate at four different p_{aH} values (3.05, 3.45, 3.55, and 3.7) showed that over this range the kinetics were independent of p_{aH} , suggesting that all the p_{aH} values at which the reaction was studied were above the 'p*K*_a' of chloroacetate at -30°C and that $k_f = k_B [B]$, where $[B]$ is the concentration of the chloroacetate ion. The average value of k_B was $0.24 \pm 0.02 \text{ l mol}^{-1} \text{ min}^{-1}$ at -30°C .

The decomposition of the intermediate in the chloroacetate solutions was so slow at -30°C that rate data were not conveniently obtained and the rate constants were measured at -20.0°C . At a p_{aH} value of 3.7 the decomposition of the intermediate was catalysed by chloroacetate over the concentration range 0.2–0.4 M with a calculated second-order rate constant, $k_s = 0.244 \text{ l mol}^{-1} \text{ min}^{-1}$.

The kinetic results obtained at low temperature are consistent with equation (1) where the intermediate is postulated to be a mixed anhydride species (2) or with schemes in which the monoester (3) is formed. To identify the intermediate further, hydroxylamine was employed as

a trapping nucleophile. When hydroxylamine (0.033 M) was added at time intervals of 5–20 min to the mixture formed on the reaction of (1) with 0.4 M chloroacetate, p_{aH} 3.7, in ethylene glycol–aqueous solution at -30°C



and the resultant solution was left for 30 min at -30°C and then warmed slowly to room temperature, yields of 21–57% of chloroacetohydroxamic acid were obtained. To establish that the species being trapped was not chloroacetic anhydride, similar trapping experiments were carried out with aniline which traps carboxylic acid anhydrides under the conditions employed. No evidence for the formation of trapping products by reaction with aniline was observed. A similar criterion had been used in our earlier work on the reaction of diphenyl sulphite with acetate at 25°C to rule out the intermediacy of acetic anhydride in that solvolysis reaction.²

In summary, from low temperature kinetic studies and nucleophilic trapping experiments we have shown that the chloroacetate ion-catalysed solvolysis of bis(*p*-nitrophenyl) sulphite (1) proceeds through nucleophilic attack by the carboxylate ion on the sulphite sulphur, resulting in intermediate formation of the mixed anhydride (2) which then decomposes further with chloroacetate catalysis. Our low temperature kinetic measurements provide the first direct spectrophotometric evidence for stepwise release of products in this reaction. These model studies may find use in the investigation of the pepsin-catalysed hydrolysis of sulphite esters.

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