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Catalytic degradation of hydrocortisone disodium phosphate solutions by copper(II) ions

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As part of a general study of the stability of hydrocortisone 21-phosphate (HDP) solutions, the effect of metal ions has been investigated. Traces of copper profoundly accelerate both the hydrolysis of the 21-phosphate ester linkage and the subsequent oxidative degradation of the dihydroxy-acetone side-chain with significant effects being observed at copper concentrations as low as 0·1 p.p.m. (w.r.t. HDP). The main degradation products are hydrocortisone, 11 β hydroxyandrost-4-ene-3,17-dione, 11 β,17α-dihydroxy-3,20-dione-4-pregnene-21-al, and 11 β, 17α-dihydroxy-3-oxo-4-etienic acid. The formation of the 21-aldehyde, a yellow, substituted glyoxal, is shown to be the cause of the undesirable yellowing of HDP solutions. The compound is known to be readily produced from the 20-keto-21-hydroxy steroid by catalytic concentrations of cupric ions (Lewbart & Mattox, 1963). The products were identified and measured by a combination of two or more of the methods of t.l.c., g.l.c., n.m.r., i.r. and u.v. spectroscopy together with elemental analysis. Copper was determined (at levels as low as 0·01 p.p.m.) by chelation and solvent extraction followed by atomic absorption spectrophotometry. Hydrolysis was measured by determining the inorganic phosphate formed, (Mokrasch, 1961) and the oxidative production of 21-aldehyde assessed from measurements of optical density at 450 nm. The copper-catalysed hydrolysis and oxidation processes are both pseudo-first order with respect to HDP concentration and the activation energy for hydrolysis (measured at 37°, 50°, 70°, 80° and 90°) is 107·0 KJ mol^{-1}. The order of both reactions with respect to copper concentration is 0·28, which strongly suggests that the hydrolysis, as the initial step in the degradation sequence, is rate-determining. No differences were shown between the degradation kinetics of submicellar and supra-micellar concentrations of HDP. The effect of antioxidants, buffers and EDTA will be briefly discussed and mechanisms proposed for the role of copper ions. Of nine ubiquitous metal ions examined, only copper, iron and nickel showed enhancement of degradation at catalytic concentrations. The effects shown by iron and nickel were only about 20 and 8%, respectively, of that produced by copper.

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Mechanism of degradation of 5-bromouracil in aqueous solutions of sodium bisulphite

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A kinetic study has been made of the reactions that occurred at 25° in solutions which contained 5-bromouracil (I) (initial concentration range 0·5–1·0 $\times 10^{-2}$ M) and sodium bisulphite (initial concentration range 0·5–6·0 $\times 10^{-1}$ M). Reactions were studied throughout the pH range 4·0–7·5 and ionic strength was maintained at 1·0 M with potassium chloride.

It has previously been reported (Sander & Deyrup, 1972) that uracil (II) was rapidly formed by reactions of I in aqueous sodium bisulphite and that II was slowly converted to

5,6-dihydrouracil-6-sulphonate (III) by the covalent addition of bisulphite ion (HSO^{3-}). We observed that II and III were both formed rapidly from I and that the relative amount of III increased as the concentration of general acids in the solution was increased. For example, the ratio of initial yields of III and II ([III]/[II]) when I reacted in a 4×10^{-2} M solution of sodium bisulphite at pH 7·0 was 1·58 when no sodium dihydrogen phosphate was present but was 4·78 when the concentration of the latter species was $3{\cdot}8 \times 10^{-1}$ M). A much slower subsequent reaction did result in the conversion of II to III.

The rates of disappearance of I and of formation of II and III were identical and were a complex function of pH and of the initial sodium bisulphite concentrations, S_T. At constant pH values the following identity related the observed pseudo-first order rate constants, k_{obsd} values, to S_T values: $k_{obsd} = C_1S_T^2/(1 + C_2S_T)$. In this identity C_1 and C_2 are constants whose values vary with the pH of the reaction solution. Typical values of C_1 and of the ratio C_1/C_2 were 0·29 M^{-2} s^{-1} and 0·07 M^{-1} s^{-1} at pH 6·5 and 0·11 M^{-1} s^{-1} and 0·025 M^{-1} s^{-1} at pH 7·5.

The results can be rationalized in terms of a reaction sequence which includes the following steps: (a) Covalent addition of HSO_3^- to I to yield 5-bromo-5,6-dihydrouracil-6-sulphonate (IV). This is a two step reaction which is acid catalysed when small concentrations of acid are present but whose rate is independent of acid concentrations when the latter are large. (b) A displacement reaction by $SO_3^=$ on the bromine atom of IV to yield an unstable enolate ion. (c) Either protonation of the enolate ion by acids to yield III or elimination of $SO_3^=$ in both spontaneous and acid catalysed reactions to yield II. The results suggest that the formation of IV from I is the rate determining process under the experimental conditions.

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Degradation of paracetamol by a *Penicillium* species

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A mould was isolated from an acidic solution of paracetamol which had been stored in the laboratory. Analysis of the paracetamol solution by polarography showed a decrease in paracetamol concentration (Porter, personal communication). The mould was subsequently identified as a *Penicillium* species.

The *Penicillium* isolate was grown at 25° in a liquid mineral salts medium containing acetamide 1% (w/v) as the carbon source. After incubation a triple washed suspension was prepared in quarter-strength Ringer solution and used to inoculate mineral salts medium (100 ml) containing paracetamol as sole carbon source at concentrations of 0·01, 0·05 and 0·1% (all w/v). The absence of paracetamol toxicity at these concentration was determined by simultaneously inoculating mineral salts media containing both paracetamol and acetamide 1% (w/v). Uninoculated controls were similarly prepared. All media were incubated at 25° on an orbital shaker. The presence or absence of growth was estimated visually and possible degradation of paracetamol measured by ultraviolet spectroscopy.

Results showed that the *Penicillium* isolate was able to utilize paracetamol at the concentrations tested. A decrease in concentration of paracetamol occurred and a shift in ultraviolet spectrum was obtained. The previously colourless paracetamol solution darkened appreciably over 14–21 days incubation and the degree of darkening was related to the initial paracetamol concentration. The metabolic products of the degradation proved to be toxic to the mould over 14–21 days incubation. Control solutions showed no change in paracetamol concentration or any visible darkening during the same period.