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Note

Spontaneous oscillations in the oxidative degradation of 2,4,6/3,5-pentahydroxycyclohexanone (*scyllo*-inosose)*

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It has been reported that 2,4,6/3,5-pentahydroxycyclohexanone (*scyllo*-inosose, **1**) functions as an efficient electron-donor in hydroxylation reactions in Tris buffer[1]. In our hands, substitution of phosphate buffers of the same pH in similar systems led to marked decomposition of the electron donor. Solutions of 2,4,6/3,5-pentahydroxycyclohexanone in phosphate at pH 7.5 became yellow and then virtually colourless, a process that was markedly accelerated in more-strongly alkaline solution. Decomposition of 2,4,6/3,5-pentahydroxycyclohexanone at alkaline pH may be inferred from the work of Heyns and Paulsen[2] on the catalytic oxidation of *myo*-inositol. When the reaction was conducted at pH 3–5, yields of 35% of reducing material were obtained, but in hydrogencarbonate-buffered solution at pH 8–9, this value fell to 7%. The present paper reports aspects of the unusual behaviour of 2,4,6/3,5-pentahydroxycyclohexanone in alkaline solutions.

Fig. 1 shows the spectral changes occurring in a phosphate-buffered solution of the cyclohexanone (**1**) from which air was excluded. From an initial absorption at 283 nm, there was an increase at 287 nm, followed by the development of a stable peak at 307 nm. The rate of development of this peak was pH-dependent (Fig. 2), the rate of increase being faster with 0.1M solutions in sodium carbonate or hydroxide. It was also dependent on buffer concentration (Fig. 2). Solutions of 2,4,6/3,5-pentahydroxycyclohexanone in arsenate behaved in a similar manner to those in phosphate, but no increase in absorbance was noted in 0.1M solutions in Tris · HCl, pH 7.5; phosphoric acid, pH ~1; sodium sulphate, pH 6.6; or sodium borate, pH 9.2. Treatment of solutions showing the absorption maximum at 307 nm with acetic acid resulted in a shift to 279 nm.

These changes in absorption are interpreted as resulting from the enolisation of 2,4,6/3,5-pentahydroxycyclohexanone (**1**), catalysed by phosphate or arsenate and favoured by higher pH. The hydroxycyclohexanone is known[3] to undergo

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*Dedicated to Professor Stephen J. Angyal on the occasion of his retirement.

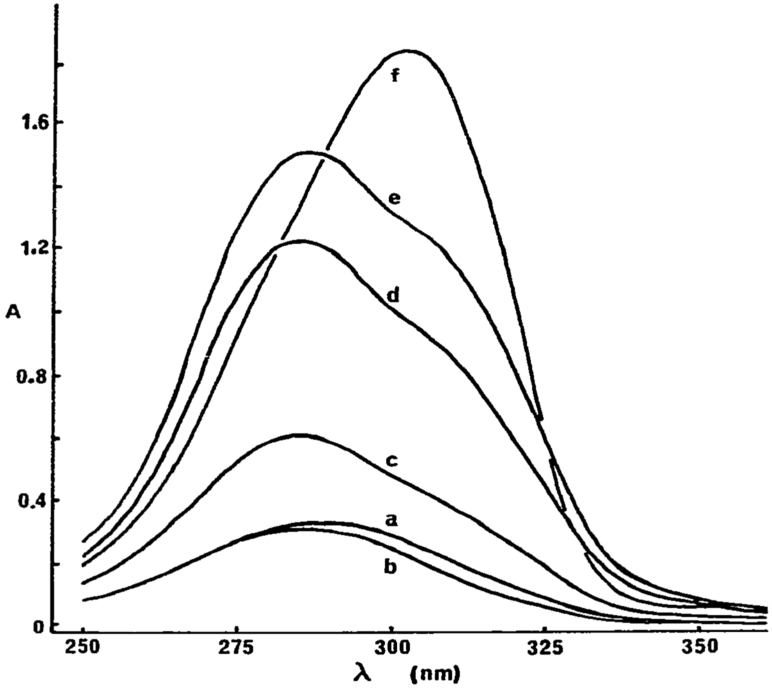


Fig. 1. 2,4,6/3,5-Pentahydroxycyclohexanone (15 μmol/mL) in oxygen-free, 0.1M potassium phosphate, pH 8.0. The solution was contained in a Thunberg cuvette under nitrogen and was scanned after: a, 0; b, 0.25; c, 1.5; d, 2.0; e, 2.5; and f, 24 h (on sample diluted 10-fold with oxygen-free buffer).

enolisation about 1000 times as rapidly as D-glucose, and the shift in absorbance-maximum from 307 to 279 nm on acidification is consistent with this interpretation. Both enolates **2** and **3** have been shown to absorb in the region of 300 nm. Heyns and Paulsen reported[2] λ_{max} of ~300 nm and $\varepsilon = 4000$ for **2** in 0.1M sodium hydroxide under (presumably) aerobic conditions, which should be compared with an ultimate value for λ_{max} of 307 nm and ε of 9000 under anaerobic conditions in the present study. Fatiadi and Isbell reported[4] λ_{max} 308 nm and ε of 17700 for an aqueous solution of the potassium salt of **3**. The nature of the initial species absorbing at 307 nm in this study is presumed to be **2**. The reason for the lower ε value reported by Heyns and Paulsen is probably found in the presumed aerobic conditions employed by those authors.

This assignment is supported by recent work of deWit and co-workers[5], who ascribed absorption at 310 nm in aqueous alkaline solutions of a series of monosaccharides to enediol-anion species and not to β-elimination intermediates. As in the present study, the absorption was eliminated on acidification and the enediol anion also underwent rapid oxidation.

When anaerobic solutions of 2,4,6/3,5-pentahydroxycyclohexanone that had reached maximal absorbance at 307 nm were exposed to oxygen, a further series of

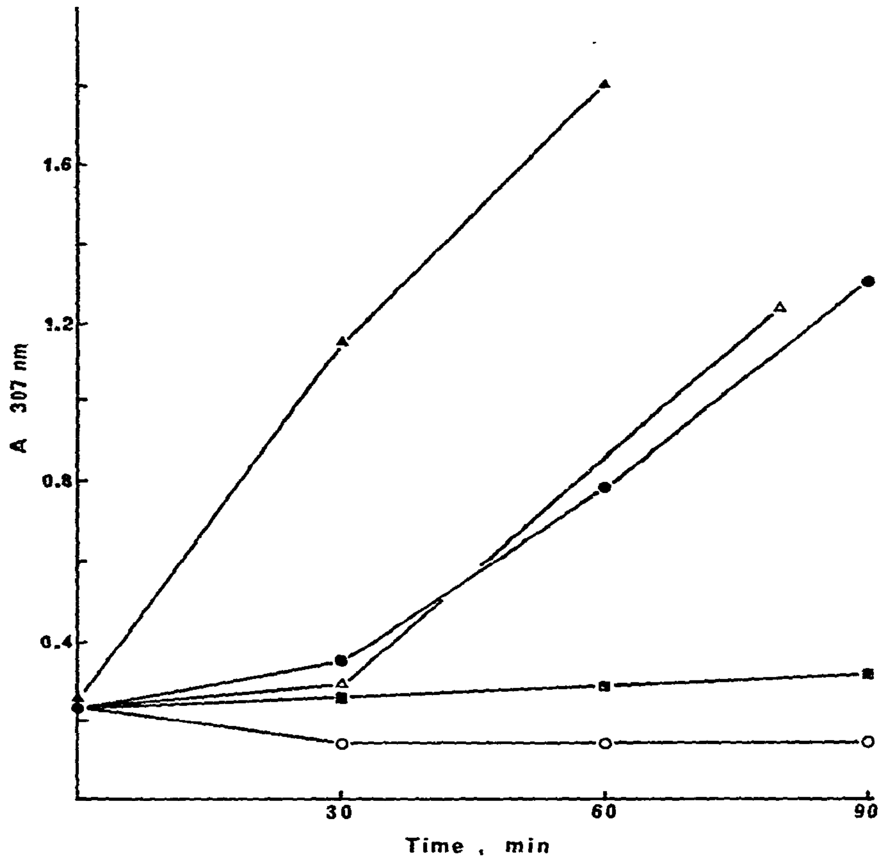
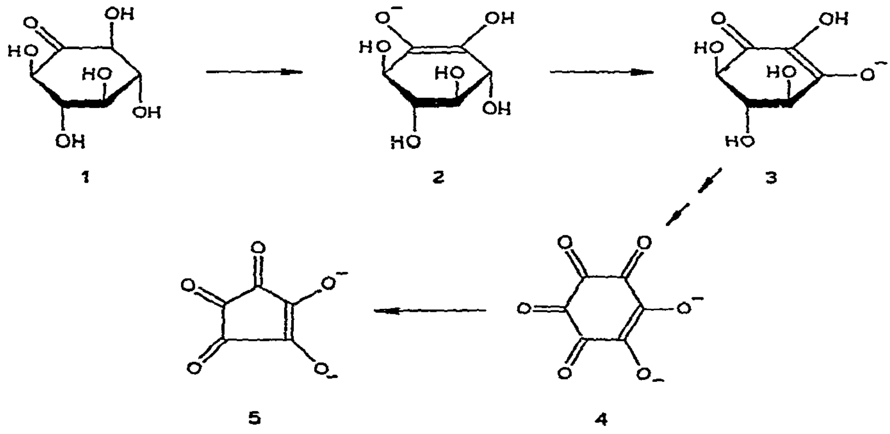


Fig. 2. Rate of increase of absorbance at 307 nm of oxygen-free solutions of 2,4,6/3,5-pentahydroxycyclohexanone (15 μmol/mL) in: ■——■, 0.1M potassium phosphate, pH 6.0; ●——●, 0.1M potassium phosphate, pH 7.5; ▲——▲, 0.1M potassium phosphate, pH 8.5; ○——○, 0.01M potassium phosphate, pH 7.5; and △——△, 0.1M sodium arsenate, pH 7.5.



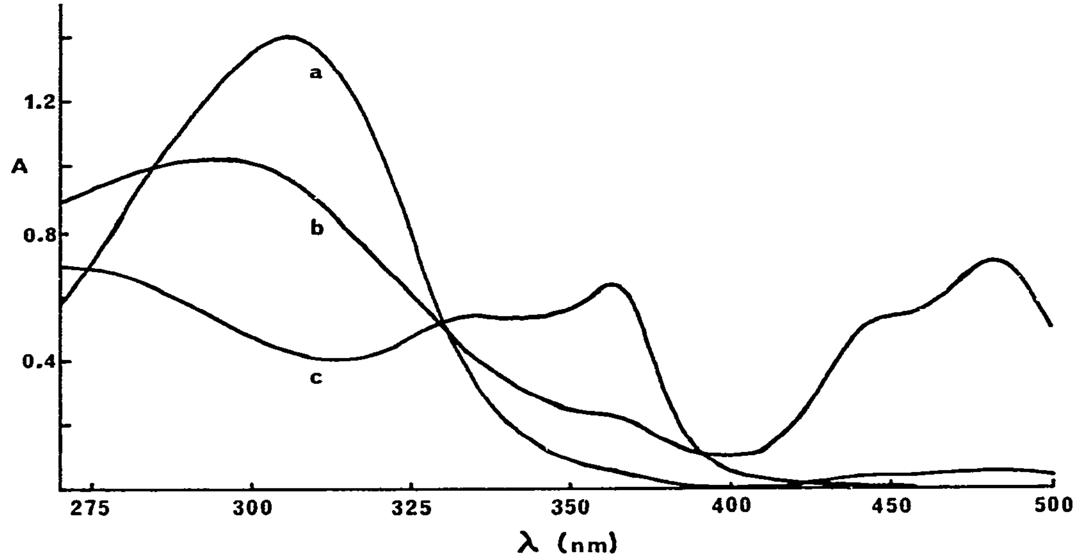


Fig. 3. Spectral changes during oxidation of 2,4,6/3,5-pentahydroxycyclohexanone (0.33 μmol/mL) in 1.0M sodium phosphate, pH 7.5; a, under anaerobic conditions in a Thunberg cuvette; b, 21 h after opening the cell to air; and c, 39 h after opening the cell to air.

spectral changes was apparent (Fig. 3). The absorbance at 307 nm decreased, and new maxima were established at 448 and 482 nm. These in turn decreased and were replaced by a peak at 363 nm with a shoulder at 333 nm. During this change, an isosbestic point was established at 395 nm. The first step in this oxidative sequence may be the formation of the enediolate **3**, absorbing at 308 nm. This compound is known to be unstable in water, the potassium salt decomposing almost completely within 30 min of dissolution[4]. Further successive enolisations and oxidations of the enediolate yield rhodizonic acid (**4**), absorbing at 448 and 484 nm, which is known[6] to give rise to the (relatively stable) croconic acid (**5**) having a maximum[7] at 365 nm. Fig. 3 provides spectral evidence for the presence of these compounds, and material isolated from oxidised solutions of **1** was indistinguishable in electrophoretic and chromatographic properties from an authentic sample of potassium croconate. By using the molar absorbance quoted from Yamada *et al.*[7] for croconate, the absorbance measured at 365 nm corresponds to a yield of only 5.2% for this compound. The low yield of croconate clearly indicates that the reaction sequence shown does not account for the major degradative reactions of **2**, although it does account for those intermediate species giving strong and characteristic u.v. spectra.

A unique feature of this oxidation is the oscillatory nature of the changes in concentration of intermediates absorbing at 307 nm. After an initial lag-phase, oscillations were observed to be either regular or random (Figs. 4 and 5), and dependent upon oxygen availability and temperature. For example, when the oxidation was performed in 0.1M sodium hydroxide, no detectable oscillations were observed

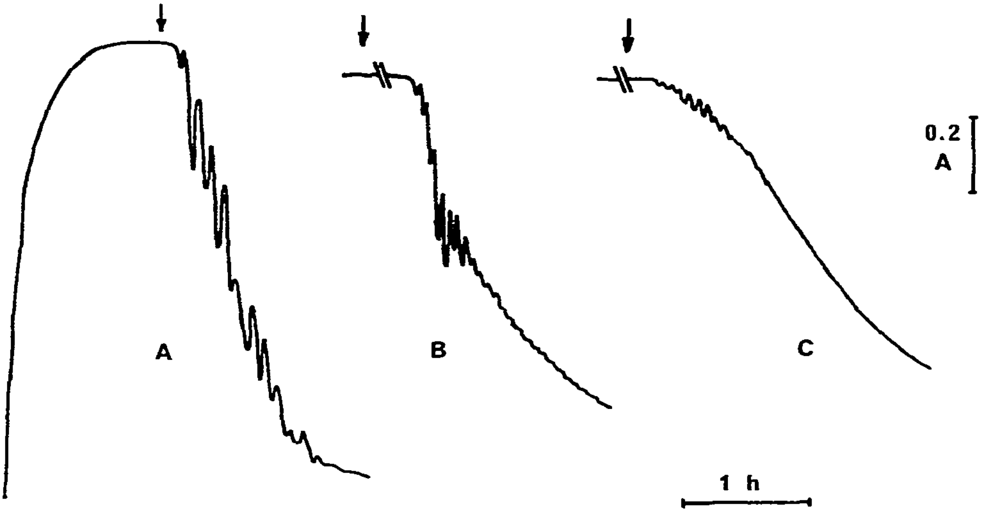


Fig. 4. Changes in absorbance at 307 nm of solutions of 2,4,6/3,5-pentahydroxycyclohexanone (0.16 μmol/mL) in: A, 0.1M sodium hydroxide; B, 0.1M potassium phosphate, pH 8.5; C, 0.1M potassium phosphate, pH 7.5. Solutions at 30° in Thunberg cuvettes under nitrogen were allowed to attain maximum absorbance and were then opened to the air (arrow).

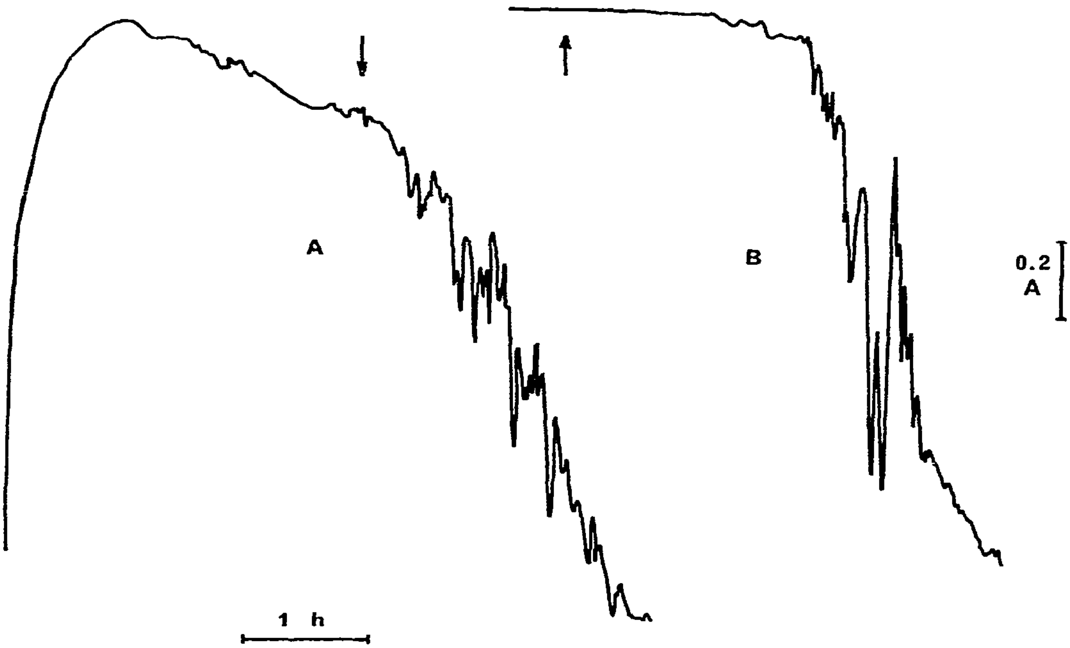


Fig. 5. Changes in absorbance at 307 nm of solutions of 2,4,6/3,5-pentahydroxycyclohexanone (0.14 μmol/mL) in: A, 0.1M sodium hydroxide; and B, 1.0M potassium phosphate, pH 8.0. Conditions were as for Fig. 4, but at 37°.

at a temperature of 20°, a regular pattern occurred at 30° (Fig. 4A), and a random pattern appeared at 37° (Fig. 5A). Accurate control of the rate of oxygen diffusion into the solution was not practical with the Thunberg cuvettes used, but it may be generally stated that, if the oxygen supply was restricted, then the rate of decrease at 307 nm was lower and any oscillations were of smaller amplitude.

Further work is necessary to provide an adequate explanation for the phenomenon, but in view of the almost universal biological distribution of *myo*-inositol[8] and accumulating evidence for the accompanying presence of 2,4,6/3,5-pentahydroxycyclohexanone, such work is warranted. The ramifications of oscillating reactions for biological systems have recently been reviewed by Hess[11], and we suggest that the foregoing reaction is worthy of further study.

EXPERIMENTAL

2,4,6/3,5-Pentahydroxycyclohexanone. — This compound was prepared by oxidation of *myo*-inositol by *Acetobacter suboxydans* and purified *via* the phenylhydrazone[12]. Spectra were recorded from a Unicam SP 800 spectrophotometer fitted with constant-temperature cell-housing and scale-expansion facility.

Procedures. — Solutions of 2,4,6/3,5-pentahydroxycyclohexanone, sodium hydroxide, and buffers were freshly prepared with oxygen-free water. Aliquots of the cyclohexanone were mixed with buffer in a Thunberg curvette to a final concentration of 0.14–15.0 μmol/mL (see legends to Figures for individual conditions). The cell was carefully evacuated and then purged with argon or nitrogen, an operation that was repeated five times. Progress of the reaction was observed by repetitive scanning or monitoring at 307 nm.

To establish the identity of croconate in oxidised solutions, aliquots were spotted with authentic samples on buffer-saturated Whatman 3MM paper and electrophoresis was performed in a solvent-cooled system at a potential gradient of 35 V per cm. Mobilities ($M_{o.g.}$) were measured relative to the anionic dyestuff "Orange G" ($M_{o.g.} = 1.0$) with D-fructose as a non-migrating marker ($M_{o.g.} = 0.0$). Buffers used and mobilities observed were as follows: 1.0M acetic acid–0.75M formic acid (pH ~1.7), rhodizonate $M_{o.g.} = 0.0$, and croconate $M_{o.g.} = 1.38$; and 0.05M citric acid adjusted to pH 5.0 with sodium hydroxide, rhodizonate $M_{o.g.} = 2.35$, and croconate $M_{o.g.} = 2.80$.

Paper chromatography conducted with 7:3 (v/v) isopropyl alcohol–water showed rhodizonate having R_F 0.08 and croconate R_F 0.06.

Rhodizonate and croconate were detected by inspection with visible and/or u.v. light at 252 nm. In each system, the presence of compounds corresponding in mobility or R_F value to rhodizonate and/or croconate confirmed the conclusions reached from the u.v. spectra of the solutions.

ACKNOWLEDGMENTS

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