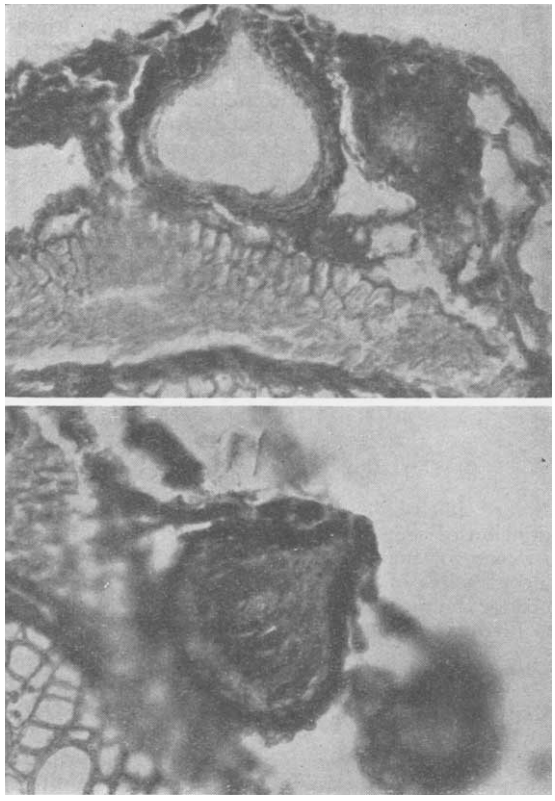

Fig. 1. Top, perithecia of *M. pinodes* treated with 0·75 per cent pentachlorophenol; bottom, normal (untreated) perithecium of *M. pinodes*

The spore counts given in Table 1 suggested that this concentration of pentachlorophenol had a powerful fungicidal effect on mature perithecia of *M. pinodes*, but that the method of application had not given complete coverage.

The second series of laboratory tests was designed to determine the effect of a range of concentrations of pentachlorophenol on 10-gm. samples which were momentarily immersed in the emulsion in order to ensure complete coverage. Spore discharge was assessed in the same way, beginning on the second day after treatment and continuing thereafter at weekly intervals for five weeks. Spore counts are given in Table 2, and these indicate a highly efficient kill of perithecia with all concentrations of pentachlorophenol down to 0·2 per cent.

After each of the first four discharge tests, the samples were allowed to dry in air. After the fifth discharge, all the samples were showered with ascospores of *M. pinodes* and incubated moist in closed containers at room temperature to determine whether recolonization by the fungus could occur at this stage. Straws untreated with pentachlorophenol but steam-sterilized were also subjected to a similar inoculation and incubation.

A very heavy mycelial growth was present on the steam-sterilized straws after incubation for five days; that on the unsterilized untreated controls was less conspicuous, possibly due to partial inhibition by strong bacterial growth. A few isolated colonies were noted on all the samples treated with pentachlorophenol except those which had received the 2 per cent treatment.

In order to examine histologically the effect of pentachlorophenol on the perithecia, representative straws from 0·75 per cent pentachlorophenol and from control samples were embedded in paraffin wax and sectioned by microtome at 10μ. The chemical completely destroyed the contents of the perithecia, which appeared either empty or with a few disorganized remains in the asci, as shown in Fig. 1 (top). Sections of normal untreated perithecia are shown in Fig. 1 (bottom).

Preliminary tests have shown that dinitro-ortho-cresol (0·2 per cent) and dinitro-secondary butylphenol (0·2 per cent) may be expected to give similar results.

Field experiments with certain of these chemicals are proceeding; but observations to date indicate that the technical difficulties of obtaining a sufficiently complete spray coverage of the pea stubble will present a serious obstacle to successful application of the method for field control of the pathogen.

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OXIDATION OF ASCORBIC ACID AND SIMILAR REDUCTONES BY NITROUS ACID

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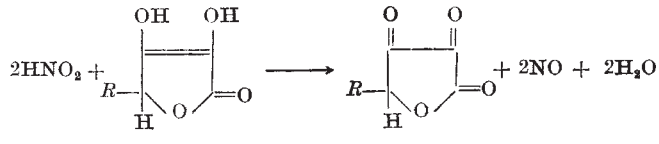
THE reaction between ascorbic acid and sodium nitrite or nitrous acid was first observed by Karrer and Bendas[1]. This reaction is general for reductones (that is to say, enediols stabilized by conjugation to a carbonyl or similar group)[2,3]; it has the characteristics of a typical oxidation-reduction process, and gives quantitative formation of nitric oxide and dehydro reductone. For example:

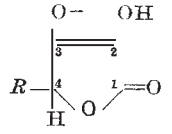


(1*a*), Ascorbic acid (R = $CH_2OH.CHOH$—)
(1*b*), Phenyl hydroxytetronic acid[2] (R = C_6H_5—)

(II*a*), (R = CH_2OH—CHOH—)
(II*b*), (R = C_6H_5—)

The optimum conditions for these reactions (in aqueous solutions at pH < 5) are similar to those in which nitrosation at nitrogen and oxygen occurs[4,5], and we sought and have found a parallel between the oxidation of reductones, and the diazotization of aromatic amines[4], and the exchange of oxygen atoms between water and nitrous acid[5].

The compounds (I*a*) and (I*b*) have characteristic absorptions[6] which are more intense than those of the dehydro reductones (II*a*) and (II*b*) and of nitrous acid, and the reaction can be followed by spectrophotometric methods. The 3-hydroxyl group of (I*a*) and (I*b*) is acidic ($pK_a \sim 4$), while that on C-2 is enolic ($pK_a \sim 12$)[7]. Thus at pH < 5 the acid (I) and its mono-anion (III) co-exist.



(III)

The kinetics of the oxidation of ascorbic acid (and to a limited extent of phenyl hydroxytetronic acid (*Ib*)) have been examined in detail at 0° C. in aqueous dioxan. A few runs were made in water, but in some conditions the reaction in water is too fast for convenient measurement. In all cases the concentration of the reductone ($0{\cdot}1$–$0{\cdot}2 \times 10^{-3}$ *M*) was much less than that of the nitrous acid ($0{\cdot}8$–$8{\cdot}0 \times 10^{-3}$ *M*).

With sodium nitrite in excess over nitrous acid (pH 3–4 in water) we observe the rate-law for reactions in both water and aqueous dioxan :

$$v = k_2 [HNO_2]^2$$

The kinetic form of an individual run is of zeroth-order in reductone and the rate is almost independent of the nature and concentration of the reductone :

(I*a*), $10^2 k_2 = 1{\cdot}7$ (mol.$^{-1}$ l.sec.$^{-1}$)
(I*b*), $10^2 k_2 = 1{\cdot}5$ (mol.$^{-1}$ l.sec.$^{-1}$)
(0° C. ; dioxan : water :: 40 : 60 v/v)

Following the interpretation given to such a kinetic form in the diazotization of the aniline[4], we conclude that the step *determining the rate* of the reaction is the formation of dinitrogen trioxide.

Reaction path A :

$$2HNO_2 \rightleftharpoons H_2NO_2^+ + NO_2^- \quad (1)$$

$$H_2NO_2^+ + NO_2^- \rightleftharpoons N_2O_3 + H_2O \quad (2)$$

$$N_2O_3 + \text{reductone} \longrightarrow \text{intermediate (IV)} \quad (3)$$

$$\text{(IV)} \xrightarrow[\text{(fast)}]{\text{nitrous acid}} \text{(II)} \quad (4)$$

In this acidity region most of the reductone is present as a mono-anion (III) ; we assume that the dinitrogen trioxide reacts rapidly with this mono-anion (step 3), to give an intermediate (IV), which then reacts rapidly with a further nitrous acid species to give the final products. (The probable nature of the intermediate is discussed below.) This kinetic scheme requires that the rate of reaction of the reductone in water should be close to that of the zeroth-order diazotization of aniline[4], and the oxygen exchange of nitrous acid via dinitrogen trioxide[5] : this is so within the limits of experimental uncertainty.

$k_2 = 0{\cdot}49$ (mol.$^{-1}$ l.sec.$^{-1}$) (oxidation of (I*a*))
$= 0{\cdot}85$ (mol.$^{-1}$ l.sec.$^{-1}$) (diazotization)[4]
$= 0{\cdot}53$ (mol.$^{-1}$ l.sec.$^{-1}$) (oxygen exchange)[5]
(0° C. ; water)

At the other extreme of our acidity-range (with $[HClO_4] \gg [HNO_2]$) we observe a different kinetic form :

$$v = k_3{}^{0H} [H^+] [HNO_2] [\text{ascorbic acid}] ;$$
$k_3{}^{0H} = 7{\cdot}5$ (mol.$^{-2}$ l.^{2} sec.$^{-1}$)
(0° C. ; dioxan : water :: 40 : 60 v/v)

(So far, this region has been explored only for the oxidation of ascorbic acid in aqueous dioxan.) With an excess of perchloric acid (about $0{\cdot}1$–$1{\cdot}0$ *M*) the concentration of ascorbate ion is very low, and we consider the mechanism to be a slow reaction between the nitrous acidium ion (V) and ascorbic acid.

Reaction path B :

$$H^+ + HNO_2 \rightarrow H_2NO_2^+ \text{ (V)}$$
$$\text{(V)} + \text{ascorbic acid} \rightarrow \text{intermediate (IV)} + H^+$$

followed by a rapid reaction of the intermediate (IV). This process has its analogy with N- and O-nitrosations, for example, the diazotization of aromatic amines in acid[4], and the acid catalysed hydrolysis of alkyl nitrites[8].

In the acidity-range between those which show mechanisms *A* and *B* there is a region in which the kinetics are of mixed order. Part of the reaction proceeds by mechanism *B*, but there is also a rate term which is second order in nitrous acid and first order in stoichiometric reductone. A similar kinetic form has been observed for reactions between nitrous acid and both aliphatic and aromatic amines[4,9]. It arises because in this region dinitrogen trioxide is in facile equilibrium with nitrous acid (steps 1 and 2 of mechanism *A*), but the proportion of the highly reactive reductonate ion is now very low ($< 0{\cdot}1$ per cent), and so the reaction with dinitrogen trioxide (step 3 of path *A*) is slow. A detailed examination of the effect of changes in acidity and nitrous acid concentration upon the reaction-rate between nitrous and ascorbic acids shows that in the region $[H^+] = 10^{-4}$–10^{-3} *M*, dinitrogen trioxide reacts with both ascorbic acid and ascorbate ion, and it is possible to estimate the relative reactivities towards dinitrogen trioxide :

$$v = [HNO_2]^2 \{k_3{}^0 [\text{ascorbic acid}] + k_3{}^- [\text{ascorbate}]\}$$

$k_3{}^0 = 73$ (mol.$^{-2}$ l.^{2} sec.$^{-1}$) ; $k_3{}^- = 1{\cdot}15 \times 10^5$ (mol.$^{-2}$ l.^{2} sec.$^{-1}$) (0° C., dioxan : water 40 : 60 v/v).

Thus we have reactions of the powerfully electrophilic nitrous acidium ion with the undissociated reductone, and of the weakly electrophilic dinitrogen trioxide with both the undissociated reductone and its more reactive anion.

Chloride and bromide ions catalyse the oxidation of ascorbic acid by nitrous acid by forming nitrosyl halides, which are themselves powerful nitrosating agents[4].

$$H_2NO_2^+ + X^- \rightleftharpoons NOX + H_2O$$

$$NOX + \begin{Bmatrix}\text{ascorbic acid}\\ \text{ascorbate ion}\end{Bmatrix} \rightarrow \text{intermediate (IV)} + \begin{Bmatrix}HX\\ X^-\end{Bmatrix}$$

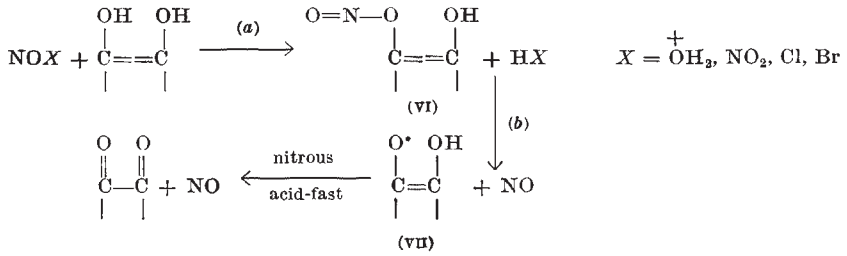
$$(X = Cl, Br)$$

From our kinetic results we can calculate specific rate-constants for the processes, and estimate the relative reactivities of various species towards dinitrogen trioxide and the nitrous acidium ion. In making these calculations we have ignored effects due to activity changes; these should not be large because the ionic strengths of the solutions are low, and approximately constant. Some of the numerical values are uncertain, because they are derived from small differences between large quantities.

We can formulate the reaction between a nitrosating agent and a reductone in various ways. Perhaps the simplest is a preliminary electrophilic attack upon the reductone (*a*), with formation of a nitrite (VI), which then decomposes (*b*).

It is, of course, possible that steps (*a*) and (*b*) may be synchronous, and the process would then be considered as a transfer of a single electron to the nitrosating agent. In either event we conclude that the first step of reaction between a nitrosating agent and a reductone, forming the semiquinone (VII) (which may be identical with the above-mentioned 'intermediate' (IV)), is followed by a rapid reaction between (VII) and nitrous acid, or some species in equilibrium with it.

It seems to us that there is, therefore, a very close similarity between this oxidation – reduction reaction and the well-characterized heterolytic nitrosations by electrophilic reagents.



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A TWO-DIMENSIONAL PAPER CHROMATOGRAPHIC METHOD COMBINING ION-EXCHANGE AND PARTITION TECHNIQUES

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ALTHOUGH the two-dimensional paper chromatographic method has been successfully applied to separations involving a wide range of substances, improvements and modifications are continually being proposed to make this technique even more discriminating.

Separations can often be improved by increasing the complexity of one or more of the conditions under which a chromatogram is developed, as, for example, in the use of three- or four-component solvent mixtures. On the other hand, the use of simple conditions to increase the reproducibility is often insufficient to effect an adequate separation. Thus, some approach between the two extremes has to be adopted. The present method, however, offers a means of increasing reproducibility in addition to separability.

This new approach to paper chromatography comes as a result of the development of a range of Whatman ion-exchange celluloses in these Laboratories. These new materials have been prepared by introducing acidic or basic groups into the cellulose molecule such that, under favourable conditions, ion-exchange involving these groups will be the dominating factor in a chromatographic separation[1]. Where, however, it is possible to suppress the activity of these groups such that ion-exchange is no longer the dominating factor, any chromatographic separations under such conditions will be largely as a result of a partition-type mechanism similar to that on unmodified cellulose. On the one material, in paper form, therefore, it is possible to combine ion-exchange and partition techniques in the form of a two-dimensional chromatogram, and this very simply is the basis of the method described below.

The ion-exchange materials investigated contained medium or weak strength functional groups such that where necessary their ion-exchange properties can be suppressed to varying extents by addition of acid or alkali to a developing organic solvent. Details of some separations investigated are as follows.

(1) Phosphorylated cellulose, containing dihydrogen phosphate groups, was used in the form of its mono-ammonium salt. A mixture of copper, manganese, cobalt, cadmium, iron, bismuth, zinc and mercury was applied as a spot near one corner of a