

The results agree well to those taken by the classical methods.

The aforesaid method cannot be used in the case of hydrogenated and oxidized fats and oils.

BIBΛΙΟΓΡΑΦΙΑ

1. A.O.C.S. Methods Cd. 4—40.
2. R.F. Goddu, N. F. Leblanc, C. M. Wricht: *Anal. Chem.* **27**, 1251 (1955)

3. Arnold Antonis: *J. Lipid Research* **1**, 485—6 (1960).
4. E. Vioque, M. P. Maza: *C. A.* **59**, 7759e (1963).
5. Wesley D. Skidmore, C. Etenman: *J. Lipid Research* **3**, 356 (1962).
6. S. Mizukami, K. Nagata: *C. A.* **56**, 8005 (1962).
7. *Organic Synthesis* **21**, 14 (1941).
8. W. J. Jonden: *Statistical Methods* **42**, (1951).

(Εισήχθη η̄ 10η Ἰανουαρίου 1965)

The mechanism of the reaction between nitrous and hyponitrous acid in acetate buffer.

By C. N. POLYDOROPOULOS and M. PIPINIS

The reaction between nitrous and hyponitrous acid over the pH range 3.98—5.30 (in acetate buffer) is very complex. The mechanism involves slow formation of N_2O , which a) catalyses the decomposition of $HN_2O_2^-$ and b) reacts with $H_2N_2O_2$. At the same time the undissociated HNO_2 exhibits the same action on both $HN_2O_2^-$ and $H_2N_2O_2$.

The reaction between nitrous and hyponitrous acid has been reported first by Thum (1) but since then it has attracted very little attention. Thum found that nitrous and hyponitrous acid interact quickly in acid solution with gas evolution and in the proportion of one molecule each. Recently, the same reaction has been investigated by Hughes and Stedman (2), also in acid solution. These authors conclude that the mechanism of the reaction involves the action of the species HNO_2 on $H_2N_2O_2$. The products of the reaction were found to be N_2O , N_2 , and nitrate, the latter being formed in amounts equivalent to the decrease of nitrite.

However, it is worth while to study the reaction under biochemically important pH conditions in view of the recent interest shown (by biochemists) in the intermediates in the «nitrogen cycle» and reactions between them. Acetate buffer is preferable because a) it does not seem to take part in the reaction b) it does not interfere with the determination of the concentration of hyponitrite and c) it covers the most important pH range.

The reaction in acetate buffer turned out to be extremely complex. This short communication is intended to bring forth only the main features of the mechanism. Considerably more results are needed if a full understanding is desirable.

Experimental

Hyponitrite was prepared by the electrolytic method (3). Solutions of $H_2N_2O_2$ were prepared as described before (4) and used immediately. Other reagents

were of A. R. purity. Only freshly prepared solutions of $NaNO_2$ were used.

Rate values reported here refer to 20.0°C. The three solutions, $H_2N_2O_2$, $NaNO_2$, and the acetate mixture, were brought to 20° and mixed quickly. The concentration of hyponitrite was followed by the standard precipitation method (5), and those of nitrite and nitrate were determined spectrophotometrically. The runs extended over a period of 2-10 hours. The ionic strength used was 0.050.

Results

1) At pH above 6 nitrite and hyponitrite do not interact. Hyponitrite decomposes at the same rate as if it were alone (see ref. 4 pp. 325-26).

2) At pH=5.30 nitrite catalyses the decomposition of hyponitrite. The concentration of nitrite remains practically constant throughout the run. A plot of $\log [H_2N_2O_2]$ against time is almost a straight line. After a few hours the slope is a little higher (negatively) than in the beginning. Values of $-d\log[H_2N_2O_2]/dt$ taken after 8 hours are: 1.50, 1.84, and 2.41×10^{-4} (min^{-1}), for a nitrite concentration: 0.5, and 8×10^{-3} respectively. Note that this slope is a linear function of the square of the concentration of nitrite.

3) At pH=3.98 the situation is entirely different. The nitrite is largely consumed during the run, and correspondingly, the slope $-d\log [H_2N_2O_2]/dt$, is reduced rapidly. The order of the reaction with respect to nitrite is between 1 and 2.

For these runs, the rate, $v = -d[H_2N_2O_2]/dt$,

was evaluated graphically. The ratio, $v/[H_2N_2O_2]$ [nitrite] against [nitrite] for constant $[H_2N_2O_2]=0.042$, is shown in Fig. 1 (circles). Other values of the same ratio for $[H_2N_2O_2]$ ranging from 0.02 to 0.05 fall very near to the line drawn (crosses).

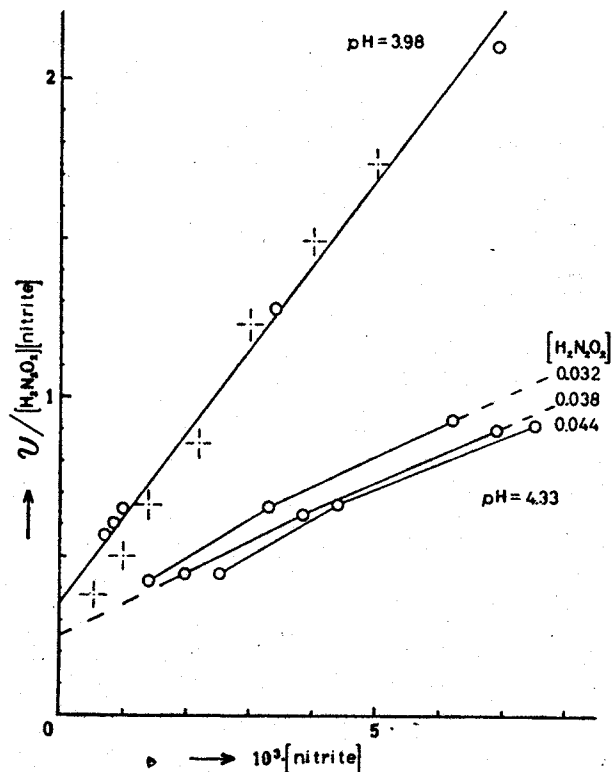


Fig. 1. Dependence of the rate on the concentrations at pH 3.98 and 4.33.

It is noticeable that the ratio of the consumptions $R=\Delta[H_2N_2O_2]/\Delta[\text{nitrite}]$ remains constant throughout a run and for all runs at the same pH. Its mean value for $pH=3.98$ is $R=5.68$.

Nitrate is formed during the reaction in amounts equivalent to the decrease of nitrite.

4) At pH values between those mentioned above, the behaviour of the reaction is intermediate. The ratio R increases with increasing pH: $R=10.52$ and 21.50 , for $pH=4.33$ and 4.67 , respectively. Note that R is a linear function of $1/(H^+)$. Nitrate equivalent to $\Delta[\text{nitrite}]$ is always formed.

At $pH=4.67$, the nitrite keeps decreasing during the run, however slowly. As a consequence, one would expect the graph of $\log[H_2N_2O_2]$ against time to be a line curved upwards. Actually it is curved a little downwards. Values of the slope $-d\log[H_2N_2O_2]/dt$ for constant $[H_2N_2O_2]=0.045$ and for the first hour of the runs are: 0.347 , 1.33 , 2.25 , 5.24 , 6.66 , and 9.42×10^{-4} (min^{-1}) for nitrite concentration: 0 , 2 , 3 , 5 , 6 , and 7×10^{-3} , respectively. It can be shown that this slope is

again a linear function of $[\text{nitrite}]^2$. However, the order of the reaction with respect to hyponitrite is less than 1 (e.g., $-d[H_2N_2O_2]/dt=1.0$ and 1.3×10^{-4} for $[H_2N_2O_2]=3.0$ and 5.25×10^{-2} respectively, and for $[\text{nitrite}]=0.008$ in both cases).

The runs at $pH=4.33$ look like those at $pH=3.98$. The graphs of $\log[H_2N_2O_2]$ against time are lines curved upwards, because of the relatively rapid decrease of nitrite. The rate, $v=-d[H_2N_2O_2]/dt$, was also estimated graphically. Some values are shown in Fig. 1. The order of the reaction is less than 1 with respect to hyponitrite and between 1 and 2 with respect to nitrite.

5) There is no appreciable change in the rate of the reaction, if the concentration of the buffer mixture is reduced by $1/2$, without a change in the pH.

Mechanism

It seems that the rate, $v=-d[H_2N_2O_2]/dt$, consists of at least two terms of which one depends on the square of the concentration of nitrite.

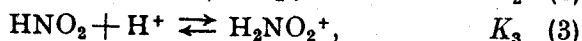
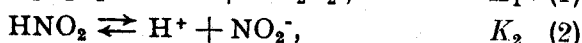
The species in equilibrium with HNO_2 are NO_2^- , $H_2NO_2^+$, N_2O_3 , etc. Of these, NO_2^- is excluded from consideration because of result 1. It can easily be shown that the equilibrium concentrations of HNO_2 and $H_2NO_2^+$ are proportional to the total nitrite concentration. Only the concentration of N_2O_3 is proportional to $[\text{nitrite}]^2$.

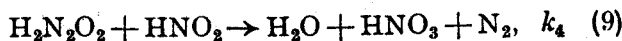
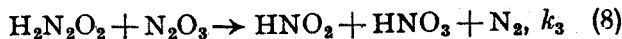
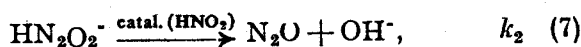
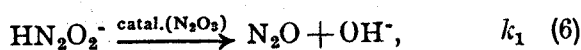
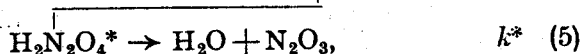
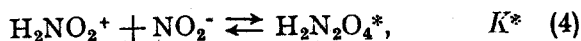
Therefore, apart from the HNO_2 already known (2) to react with $H_2N_2O_2$, the action of N_2O_3 on hyponitrite must be considered. On the other hand, the change of the value of R with pH suggests that there are at least two parallel reactions involving $H_2N_2O_2$ and $HN_2O_2^-$, respectively, the second of which is favoured at higher pH values and does not destroy nitrite.

A mechanism involving N_2O_3 in equilibrium concentration has been tried unsuccessfully. It seems that the formation of N_2O_3 can not be assumed as fast enough over all the pH range considered. The results at $pH=5.30$ and 4.67 suggest a rather long induction period for the formation of N_2O_3 .

Another mechanism, involving the action of N_2O_3 in a steady state concentration and overlooking the action of HNO_2 , has also been proved inadequate.

It seems, therefore, that the mechanism involves at least the following reactions and equilibria:





The equilibrium constants and rates are defined as follows:

$$K_1 = \frac{(\text{H}^+)[\text{HN}_2\text{O}_2^-]}{[\text{H}_2\text{N}_2\text{O}_2]}, \quad K_2 = \frac{(\text{H}^+)[\text{NO}_2^-]}{[\text{HNO}_2]}$$

$$K_3 = \frac{(\text{H}^+)[\text{HNO}_2]}{[\text{H}_2\text{NO}_2^+]}, \quad K^* = \frac{[\text{H}_2\text{NO}_2^+][\text{NO}_2^-]}{[\text{H}_2\text{N}_2\text{O}_4^]}$$

$$v^* = k^*[\text{H}_2\text{N}_2\text{O}_4^*] = \frac{k^*}{K^*}[\text{H}_2\text{NO}_2^+][\text{NO}_2^-]$$

$$v_1 = k_1[\text{HN}_2\text{O}_2^-][\text{N}_2\text{O}_3] = \frac{k_1 K_1}{(\text{H}^+)}[\text{H}_2\text{N}_2\text{O}_2][\text{N}_2\text{O}_3]$$

$$v_2 = k_2[\text{HN}_2\text{O}_2^-][\text{HNO}_2] = \frac{k_2 K_1}{(\text{H}^+)}[\text{H}_2\text{N}_2\text{O}_2][\text{HNO}_2]$$

$$v_3 = k_3[\text{H}_2\text{N}_2\text{O}_2][\text{N}_2\text{O}_3]$$

$$v_4 = k_4[\text{H}_2\text{N}_2\text{O}_2][\text{HNO}_2]$$

The brackets stand for concentrations, whereas (H^+) is the hydrogen ion activity.

In the pH range considered, the analytically determined hyponitrite is virtually equal to $[\text{H}_2\text{N}_2\text{O}_2]$, because the ratio, $[\text{HN}_2\text{O}_2^-]/[\text{H}_2\text{N}_2\text{O}_2]$, is negligible, and the total nitrite concentration is $[\text{nitrite}] = [\text{NO}_2^-] + [\text{HNO}_2]$.

By the appropriate substitutions one finds:

$$v^* = x^* \frac{[\text{nitrite}]^2}{\left(1 + \frac{K_2}{(\text{H}^+)}\right)^2}, \quad \text{where } x^* = \frac{K_2 k^*}{K_3 K^*}$$

For a value of $K_2 = 5.5 \times 10^{-4}$ ($5.5 = 4.5/0.82$ where 0.82 is the activity coefficient of NO_2^- for $\mu = 0.05$) it can be calculated that v^* (the rate of formation of N_2O_3) at $\text{pH} = 5.30$ must be about 300 times less than it is at $\text{pH} = 4.00$, for constant $[\text{nitrite}]$. This can explain the long induction period at high pH values (compare result 2).

According to the assumed mechanism, the rates of decrease of hyponitrite and nitrite would be:

$$-\frac{d[\text{H}_2\text{N}_2\text{O}_2]}{dt} = v = v_1 + v_2 + v_3 + v_4$$

$$-\frac{d[\text{nitrite}]}{dt} = v_3 + v_4$$

By division and substitution of the v 's one has

$$\frac{d[\text{H}_2\text{N}_2\text{O}_2]}{d[\text{nitrite}]} = \frac{\left(\frac{k_1 K_1}{k_3 (\text{H}^+)} + 1\right) k_3 [\text{N}_2\text{O}_3] + \left(\frac{k_2 K_1}{k_4 (\text{H}^+)} + 1\right) k_4 [\text{HNO}_2]}{k_3 [\text{N}_2\text{O}_3] + k_4 [\text{HNO}_2]}$$

If it happened that $k_1/k_3 \approx k_2/k_4$ (which does not seem highly improbable) one would have practically:

$$\frac{d[\text{H}_2\text{N}_2\text{O}_2]}{d[\text{nitrite}]} \approx \frac{k_1 K_1}{k_3 (\text{H}^+)} + 1 \approx \frac{k_2 K_1}{k_4 (\text{H}^+)} + 1 \approx \frac{d[\text{H}_2\text{N}_2\text{O}_2]}{d[\text{nitrite}]} = R$$

This would explain why R is found to be a linear function of $1/(\text{H}^+)$. The value of the intercept is indeed found to be very near to 1. This is also in agreement with earlier investigations of the same reaction in acid solution (1,2) where R is reported to be about 1.

At low pH values, the formation of N_2O_3 may be assumed to be rapid enough. If so, the concentration of N_2O_3 is in equilibrium with HNO_2 ,



$$K_5 = \frac{[\text{N}_2\text{O}_3]}{[\text{HNO}_2]^2}, \quad \text{and } [\text{N}_2\text{O}_3] = K_5 [\text{HNO}_2]^2 \quad (11)$$

and the rate, as defined above, can be expressed by (12)

$$\frac{v}{[\text{H}_2\text{N}_2\text{O}_2]} = k_4 \frac{R_{24}}{F} [\text{nitrite}] + k_3 K_5 \frac{R_{13}}{F^2} [\text{nitrite}]^2 \quad (12)$$

$$\text{where } R_{13} = 1 + \frac{k_1 K_1}{k_3 (\text{H}^+)}, \quad R_{24} = 1 + \frac{k_2 K_1}{k_4 (\text{H}^+)},$$

$$\text{and } F = 1 + \frac{K_2}{(\text{H}^+)}.$$

Fig. 1 seems to satisfy eq. (12) for $\text{pH} = 3.98$.

At intermediate pH values, N_2O_3 is very likely to attain a steady state concentration as soon as $v = v_3$. Then

$$[\text{N}_2\text{O}_3] = \frac{x^* [\text{nitrite}]^2}{k_3 F^2 [\text{H}_2\text{N}_2\text{O}_2]} \quad (13)$$

If $[\text{N}_2\text{O}_3]$ is given by (13) one finds for the overall rate, v , the relationship (14).

$$\frac{v}{[\text{H}_2\text{N}_2\text{O}_2]} = k_4 \frac{R_{24}}{F} [\text{nitrite}] + x^* \frac{R_{13}}{F^2} \frac{[\text{nitrite}]^2}{[\text{H}_2\text{N}_2\text{O}_2]} \quad (14)$$

Fig. 1 shows that (14) is applicable at $\text{pH} = 4.33$. The slope of the function, $v/[\text{H}_2\text{N}_2\text{O}_2][\text{nitrite}]$ against $[\text{nitrite}]$, increases with decreasing $[\text{H}_2\text{N}_2\text{O}_2]$ whereas the intercept remains constant.

Conclusion.

The reaction between nitrite and hyponitrite at pH above 4 is highly complex. The mechanism suggested here is the simplest mechanism compatible with experiment that one can consider. The true mechanism might be even more complex. To elucidate the details and allow for an estimation of the values of the constants involved, a great deal of data is required. Such work is bound to be lengthy. Nevertheless, it is hoped to be carried out because of the importance of knowing the values of these constants for an understanding of the behaviour of nitrous acid in other reactions too.

Acknowledgement: The authors gratefully acknowledge the support of this work by the Royal Hellenic Research Foundation.

Π Ε Ρ Ι Λ Η Ψ Ι Σ

Ο μηχανισμός της αντίδρασης μεταξύ νιτρώδους και ύπονιτρώδους οξέος εντός οξικού ρυθμιστικού

Υπό Κ.Ν. ΠΟΛΥΔΩΡΟΠΟΥΛΟΥ και Μ. ΠΙΠΙΝΗ

Έρευνάται η πολύπλοκος αντίδρασις μεταξύ νιτρώδους και ύπονιτρώδους οξέος εις την περιοχήν pH 3.98 - 5.30 (έντός οξικού ρυθμιστικού μίγματος). Εις υψηλότερας τιμάς pH δέν παρατη-

(Laboratory of Physical Chemistry of the University of Athens. Solonos 104, Athens - 144)

ρείται αντίδρασις. Το νιτρώδες καταναλίσκεται σημαντικώς κατά την αντίδρασιν εάν το pH είναι χαμηλόν, αλλά η αναλογία $\Delta[H_2N_2O_2]/\Delta[nitr.]$ αύξάνει μετά του pH , ούτως ώστε εις $pH=5.30$ το νιτρώδες παραμένει πρακτικώς σταθερόν. Αι ταχύτητες εξαρτώνται γενικώς από την πρώτην και δευτέραν δύναμιν της συγκεντρώσεως του νιτρώδους, όπερ άποκαλύπτει ότι εις την αντίδρασιν λαμβάνει μέρος και το N_2O_2 εκτός του HNO_2 . Άλλά το N_2O_2 δέν εύρίσκεται εις ίσορροπίαν, ειμή μόνον εις $pH < 4$. Εις την έρευνομένην περιοχήν pH η ταχύτης σχηματισμού του N_2O_2 είναι σχετικώς μικρά, τούτο δέ κυρίως περιπλέκει την αντίδρασιν, ούτως ώστε η ταχύτης να εξαρτάται εκ των συγκεντρώσεων κατά σχέσιν πολύπλοκον ως η (14). Ούτως ό μηχανισμός φαίνεται ότι περιλαμβάνει πολλάς έξ ίσου βραδείας αντιδράσεις ήτοι τάς (5) έως (9).

REFERENCES

1. Thum A.: *Monatsh. Chem.* 14, 294 (1893).
2. Hughes M.N. and Stedman G.: *J. Chem. Soc.* 1963, 4230.
3. Polydoropoulos C. N.: *Chemistry and Industry* 1963, 1686.
4. Polydoropoulos C. N. and Pipinis M.: *Z. Physik. Chemie N. F.* 40, 322 (1964).
5. Polydoropoulos C. N. and Pipinis M.: *Chimika Chronika* 28 A, 107 (1963).

(Received on 20th January, 1965)