Kinetics and Mechanism of Reserpine Oxidation by Nitrous Acid

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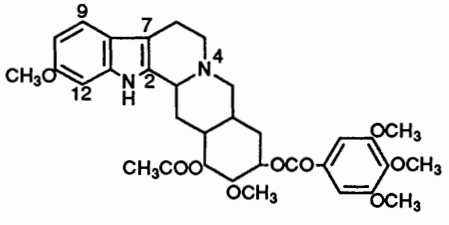
The kinetics of oxidation of the Rauwolfia alkaloid reserpine to 3,4-dehydroreserpine by nitrous acid have been measured in methanol–water media at various temperatures. The reactions are found to obey the following rate law:

$$\text{rate} = \frac{a[\text{Reserpine}]_{tot}[\text{Nit}]_{tot}[H^+]}{1 + b[\text{Nit}]}$$

where $a = 1.056$ dm^6 mol^{-2} s^{-1} and $b = 139$ dm^3 mol^{-1} at 25 °C in 20% methanol–water media. The presence of variable amounts, up to 1×10^{-2} mol dm^{-3} of the nucleophiles Cl^-, Br^- and SCN^- in the reaction media has no effect on the reaction rates. This kinetic behaviour has been rationalized on the basis of a mechanism involving the formation, in a first equilibrium step, of the isolated intermediate N^1-nitrosoreserpine which rearranges in a further acid-catalysed step to give the final product.

The reaction of reserpine (**1**), the primary active alkaloid of Rauwolfia Serpentine, with nitrous acid produces a yellow-green fluorescent product (3,4-dehydroreserpine). The subsequent photometric or fluorimetric determination of this product is a well-known pharmaceutical analytical procedure.[1,2] It has been reported to be specific for alkaloids having an 11-methoxytetrahydro-β-carboline nucleus.[3]



Reserpine (**1**)

Although this analytical method is widely utilized, little information has appeared in the literature concerning the kinetics and mechanism of this reaction. In the only reported kinetic investigation of the reserpine–nitrous acid reaction, Haycock *et al.*[4] pointed out that the reaction follows a two-step mechanism and is acid-catalysed. However, the authors were unable to determine the reaction mechanism in more detail, the nature of the possible intermediates and the origin of the acid dependence on the reaction rates.

Recently, we undertook an investigation to study the kinetics and mechanisms of the more characteristic reactions of the tetrahydro-β-carboline ring. It therefore seemed of interest to re-examine the title reaction to get a deeper insight into the mechanism. On the other hand, nitrosation reactions of indole derivatives have recently attracted attention from a kinetic point of view, and some of their mechanistic aspects are still under investigation.[5–8]

Experimental

Reagents.—Reserpine was purchased from the Sigma Chemical Co. and was used as received. Stock solutions of *ca.* 1×10^{-3} mol dm^{-3} were prepared in methanol and stored in the dark to avoid photo-decomposition. N^1-Nitrosoreserpine was prepared in the same manner as other N^1-nitrosoindole derivatives[9] by treatment of reserpine with an excess of sodium nitrite under very mild acidic conditions (acetic acid–acetate buffers, pH *ca.* 4–5). It was identified by elemental analysis (Found: C, 62.0; H, 6.3; N, 6.4; O, 25.35. $C_{33}H_{39}N_3O_{10}$ requires: C, 62.10; H, 6.11; N, 6.58; O, 25.09) and IR (absence of NH absorption band). N^4-Methylreserpine sulphate was prepared by literature methods[10] and was used for comparison purposes. Sodium nitrite, hydrochloric acid and all other reagents were AnalaR grade (Merck) and were used without further purification.

Kinetic Measurements.—Kinetic measurements were performed by monitoring the absorbances of the reaction mixtures with time at the absorption maxima of the reaction product (380 nm) with a LKB Ultrospec Plus Spectrophotometer connected to a Multitech Personal Computer to control the spectrophotometric system and the uptake and processing of the data. The temperature control was better than ±0.1 °C.

Kinetic runs were carried out under pseudo-first-order conditions in solutions containing sodium nitrite and hydrochloric acid in excess over the alkaloid. Pseudo-first-order rate constants, k_{obs}, were obtained by a non-linear least-square fitting of the absorbance–time data to eqn. (1), where A_0, A_∞ and k_{obs} are floating parameters. Usually an excellent

$$A_t = A_\infty + (A_0 - A_\infty)\exp(-k_{obs}t) \qquad (1)$$

agreement between experimental and calculated A_t values was obtained for *ca.* 3–4 half lives (standard deviation of fitting $< \pm 2.0 \times 10^{-6}$). Rate constants in duplicate runs were reproducible to within better than 6%.

In some instances, when the reaction rates were very slow, a short induction period was observed. However, when this was eliminated, the above data treatment was fully satisfactory.

Results

The results of the kinetic experiments at 25 °C are collected in Table 1. The rate constants reported in this table are the averages, at least, of two independent runs.

In 20 and 30% v/v methanol–water the reaction rates increase linearly with acid concentration. Plots of k_{obs} *vs.* $[H^+]$ give straight lines passing through the origin. In contrast, plots of k_{obs} *vs.* $[\text{Nit}]_{tot}$, ($[\text{Nit}]_{tot} = [HNO_2] + [NO_2^-]$), at fixed acid

concentrations are curved. This non-linear behaviour is suggestive, as previously pointed out Haycock *et al.*[4] of reserpine–nitrous acid complex formation. Therefore, the rate law [eqn. (2)] proposed by these authors can be assumed

$$k_{obs} = \frac{a[Nit]_{tot}[H^+]}{1 + b[Nit]_{tot}} \quad (2)$$

for the initial analyses of the kinetic results. Eqn. (2) can be rearranged to give eqn. (3). The plots of $[H^+]/k_{obs}$ *versus* the

$$[H^+]/k_{obs} = (a^{-1}\cdot[Nit]_{tot}^{-1}) + b/a \quad (3)$$

reciprocal of the nitrite concentrations (Fig. 1), confirm the consistency of eqn. (2). The values of the kinetic parameters a and b for the different proportions of methanol and at different temperatures are reported in Table 2. It can be seen that a decreases with increasing methanol proportion, whereas the reverse is true for b. Also, the parameter a increases when the temperature is increased, while the opposite behaviour is observed for the parameter b.

On the other hand, since it is known that many reactions involving nitrous acid as reagent are subject to catalysis by nucleophilic species,[11] we have also investigated the influence of Cl^-, Br^- and SCN^- on the reaction rates. These ions were all added as sodium salts and their concentrations varied up to 1×10^{-2} mol dm^{-3}. The results showed the non-existence of any catalytic effect and therefore they have not been reported.

Table 1 Pseudo-first-order rate constants for the reserpine–nitrous acid reaction at 25.0 °C with different proportions of methanol ([reserpine], 1.0×10^{-4} mol dm^{-3})

$[Nit]/10^{-3}$ mol dm^{-3}	[HCl]/mol dm^{-3}	$k_{obs}/10^{-4}$ s^{-1}
20% v/v Methanol–water		
1	0.150	1.38
	0.300	2.72
2	0.150	2.49
	0.300	5.02
	0.450	7.76
	0.600	10.5
4	0.075	2.07
	0.150	4.11
	0.300	8.12
	0.450	12.1
	0.600	16.8
5	0.150	4.40
	0.300	8.82
6	0.150	5.14
	0.300	10.2
8	0.150	5.68
	0.300	12.2
10	0.150	6.47
	0.300	13.0
30% v/v Methanol–water		
1	0.600	3.30
2	0.600	5.56
4	0.150	2.21
	0.300	4.36
	0.450	6.52
	0.600	8.74
	0.800	11.8
6	0.600	11.1
8	0.600	12.3
10	0.600	13.6

Discussion

The reaction of reserpine with nitrous acid follows a similar pattern to that of the reserpine–peroxodisulphate reaction previously studied by us.[12] The major differences between both reactions are the first-order dependence on acid concentration of the former and the nature of the final products. Thus, while reserpine oxidation by peroxodisulphate takes place in two kinetically equivalent steps yielding first 3,4-dehydroreserpine and then lumireserpine (tetradehydroreserpine), the reserpine oxidation by nitrous acid gives only the dehydro derivative.

The different dependence on acid concentration of both reactions is more relevant from a mechanistic point of view. At

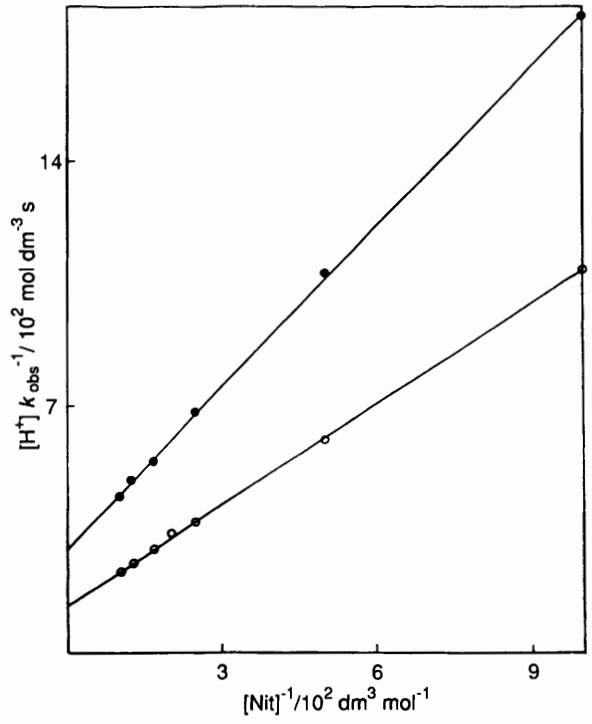


Fig. 1 Plots of $[H^+]k_{obs}^{-1}$ *vs.* the $[Nit]_{tot}^{-1}$ for the reserpine–nitrous acid reaction at 25 °C. Methanol–water: ●, 30%, $[H^+]$ 0.6 mol dm^{-3}; ○, 20%, $[H^+]$ 0.3 mol dm^{-3}.

Table 2 Kinetic parameters for the reserpine–nitrous acid reaction at different temperatures and methanol proportions

	T/°C				
	20	25	30	35	E_A/kJ mol^{-1}
20% Methanol–water					
a/dm^6 mol^{-2} s^{-1}	0.754	1.056	1.374	1.751	41.8
b/dm^3 mol^{-1}	169	139	115	84	−34.2
30% Methanol–water					
a/dm^6 mol^{-2} s^{-1}	—	0.65	—	—	—
b/dm^3 mol^{-1}	—	195	—	—	—

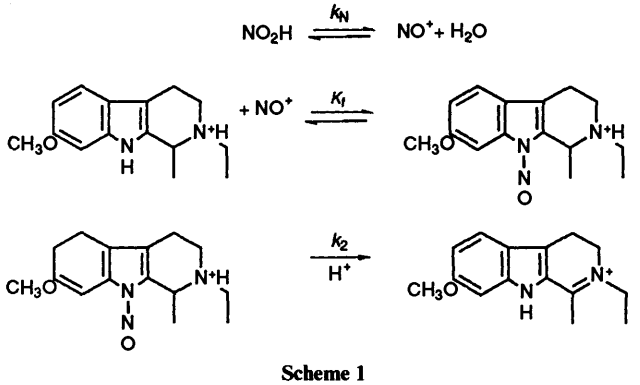
this point it is worth pointing out that this acid dependence cannot be related to any acid–base equilibria involving reserpine, which take place in highly acidic media[13] (indole ring protonation) or almost neutral solutions[14] (piperidinic nitrogen-atom protonation). Therefore, under the acidic conditions employed in the kinetic measurements, reserpine exists in the reaction media only as the monoprotonated piperidinic cation.

On the other hand, the reserpine–peroxodisulphate reaction is thought to proceed *via* 2,3-dimethyl-3*H*-indol-3-yl sulphate intermediate which further rearranges to give 3,4-dehydroreserpine. On the basis of a similar mechanism, the simplest interpretation of the first-order acid dependence in the reserpine–nitrous acid reaction might be the existence of the preequilibrium step $HNO_2 + H^+ \rightleftharpoons NO^+ + H_2O$, necessary in order to generate NO^+, the effective nitrosating agent.[11,15]

However, this hypothesis for the acid dependence does not account for the experimental results, since a limiting rate at high acid concentration should be expected. On the contrary, linear relationships between k_{obs} and $[H^+]$ are always observed over the wide range of acidity studied. The experimental results therefore point to a mechanism involving a subsequent acid catalysed step further to the reserpine–nitrous acid complex formation. Under very mild acidic conditions (acetic/acetate buffers, pH *ca.* 5) no dehydroreserpine is formed but instead a yellow product identified as N^1-nitrosoreserpine is recovered; these facts would seem to confirm this mechanism. Nitration of reserpine under similar conditions is also known to occur predominantly at this atom.[16]

The fact that N^1-nitrosoreserpine is the intermediate product in the reserpine–nitrous acid reaction is supported by the observation that this compound spontaneously decomposes in acid media to yield dehydroreserpine. This reaction is first order in acid concentration with a second-order rate constant for this process, at 25 °C and in 30% v/v methanol–water, of 3.37×10^{-3} dm^3 mol^{-1} s^{-1}. It is also worth pointing out that N^4-methylreserpine reacts with nitrous acid to yield the corresponding N^1-nitroso derivative too, but this compound does not rearrange to its dehydro derivative in acid media.

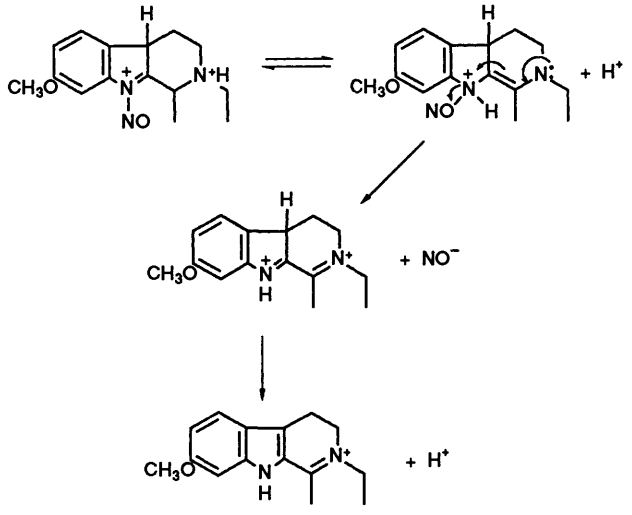
The reserpine–nitrous acid reaction can therefore be rationalized by means of the two-step mechanism depicted in Scheme 1.



Scheme 1

However, this mechanism as previously considered in the nitrosation reactions of other indolic derivatives might be an oversimplification. Thus, although the nitrosation reaction has been considered as involving only one step, it is conceivable that an initial electrophilic attack by NO^+ at C-7, the centre of greatest electronic density of the indolic moiety of reserpine,[17] takes place. An internal rearrangement of the electrophile from C-7 to N-1 could then take place. Either the electrophilic attack or the rearrangement step could be rate determining.[7,8] The lack of catalysis by Cl^-, Br^- or SCN^- nucleophiles suggests that the formation of nitrosoreserpine might be controlled by the C-7 to N-4 rearrangement. We now turn our attention to the denitrosation–aromatization step. As for denitrosation reactions of typical indole derivatives, the acid dependence of this process can be explained in terms of a rate-controlling step involving proton transfer to the substrate.[6,8] However, in the present case the presence of the tetrahydro-β-carboline nucleus in the reserpine skeleton yields a different reaction pattern; denitrosation is accompanied by aromatization of this nucleus. Furthermore, it seems evident that this process might be assisted by an electron pair from the N-4 atom, since N^4-methylreserpine, in which this electron pair is not easily available, does not aromatize.

All these requirements can be explained by considering that this process involves protonation of reserpine at C-7 and the establishment of the imino–enamino tautomeric equilibrium shown in Scheme 2. Dehydroreserpine could easily be formed



Scheme 2

from enamine rearrangement. Protonation at C-7 and shift of this indoleninic cation to its enamine form have been demonstrated in concentrated sulphuric acid solutions of reserpine.[13,18]

The mechanism shown in Scheme 1 yields eqn. (4) which is

$$\text{rate} = \frac{k_2K_1K_N[\text{Reserpine}]_{tot}[\text{Nit}]_{tot}[H^+]^2}{1 + k_2/k_{-1}[H^+] + K_1K_N[\text{Nit}]_{tot}[H^+]} \quad (4)$$

derived from the steady-state concentration of nitrosoreserpine and represents the actual concentration of alkaloid: [reserpine] = $[\text{reserpine}]_{tot}$ − [N^1-nitrosoreserpine]. Eqn. (4) can be identified with the experimental eqn. (2) provided that $1 \ll (k_2/k_{-1}[H^+] + K_1K_N[\text{Nit}]_{tot}[H^+])$. From this case, and by identifying the coefficients of both equations, the relations $a = k_1K_N$ and $a/b = k_2$ result. From the latter equality the values for k_2 at 25 °C of 7.60×10^{-3} and 3.33×10^{-3} dm^3 mol^{-1} s^{-1} in 20% and 30% v/v methanol–water media, respectively, are obtained. The very good agreement between k_2 and the value obtained in the direct reaction of N^1-nitrosoreserpine should be noted. Individual values of k_1 cannot be obtained due to a lack of information about K_N values in methanol–water media. A value of 3.0×10^{-7} dm^3 mol^{-1} has been reported for this constant in water at 25 °C.[19] A value for k_1 of *ca.* 10^7 dm^3 mol^{-1} s^{-1} is to be expected. However, as has been previously noted, k_1 is probably better related to the C-7 to N-4 rearrangement of the electrophile than with direct electrophilic attack of the nitrosonium cation; it is therefore a combination of rate and equilibrium constants.

Acknowledgements

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