Kinetics of the Oxidation of Reserpine with Peroxodisulphate

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The kinetics of the reaction between reserpine and potassium peroxodisulphate in acid media have been investigated. The results show two first-order (or pseudo-first-order) steps occurring in series. At the first step, 3-dehydroreserpine, a yellowish green fluorescence product, is obtained, which is afterwards oxidized to 3,5-tetradehydroreserpine, a product with blue fluorescence. These results have been compared with those obtained for other *Rauwolfia* alkaloids and oxidizing agents.

Reserpine (**1**) is the primary active alkaloid of *Rauwolfia serpentina*.[1,2] Because it is an important therapeutic agent, many analytical procedures have been developed for the determination of reserpine concentrations in bulk and in tablet formulations. These include the conversion of reserpine into a fluorescent derivative, 3-dehydroreserpine, with nitrous acid,[3–5] toluenesulphonic acid,[6] or vanadium pentaoxide.[7]

However, the stability of the dehydro derivative has been questioned. While several authors referred the suppression of maximum fluorescence to a quenching effect,[5,7] others[8] have observed that the maximum in the u.v. region fades away and it is replaced by a new maximum. According to the latter authors,[8] dehydroreserpine (**2**) is subsequently oxidized to tetradehydroreserpine (**3**).

The use of a stronger oxidant could confirm the existence of an additional reaction after dehydroreserpine is formed. Because of the ability of peroxodisulphate as an oxidant of indole alkaloids[9] it has been used at the present work. On the other hand, the yohimbine and reserpiline oxidation by peroxodisulphate has been studied in a previous paper.[9] Bearing in mind that the main important structural difference among these alkaloids is the position of the methoxy group on the indole ring, it should be possible to ascertain the influence of the methoxy group on the reactivity pattern.

Experimental

Reagents.—Reserpine was supplied by C. H. Boehringer Sohn and was used as received. A stock solution (10^{-4}M) was prepared by weighing. The sample was dissolved in 50% v/v methanol–water. Potassium peroxodisulphate and sulphuric acid were AnalaR grade chemicals and were not purified before use.

Kinetic Data.—Kinetic runs were carried out in 1 cm silica cells in the thermostatted cell compartment of a Perkin–Elmer Lambda-5 spectrophotometer. The temperature in the cell was fixed at 20.0, 25.0, 30.0, 35.0, and 40.0 ± 0.1 °C. These kinetic runs were made in solutions containing constant acid concentration ([H_2SO_4] 0.5M) and an excess of peroxodisulphate ions by following the change in absorbance at 383 nm at fixed time intervals. At this wavelength only 3-dehydroreserpine absorbs. During the course of the reaction, the absorbances at this wavelength increased first, finally to decrease with time. The rate constants were obtained by non-linear least-squares fitting[10] of absorbances *versus* time measurements to equation

$$A = \frac{k_{1obs}\,A_t}{(k_{2obs} - k_{1obs})\varepsilon}\,[\exp(-k_{1obs}t) - \exp(-k_{2obs}t)] \quad (1)$$

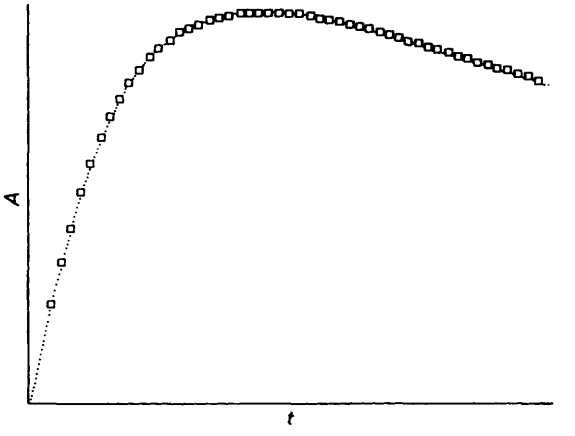


Figure 1. Plot of absorbance against time

(1) in which A_t (the total concentration of the alkaloid), ε (the molar absorption coefficient of dehydroreserpine), and the rate constants, k_{1obs} and k_{2obs}, were taken as floating parameters. A smooth deviation of the absorbance values to equation (1) was observed at early times (< 1 min). However, when this deviation was deleted an excellent agreement between experimental and calculated A values was obtained. Since A is represented as the difference of two exponential functions, it will, as expected, go through a maximum (see Figure 1). The method always gave reproducible results, and rate constant errors in duplicate and triplicate runs were estimated to be less than 6%.

The temperature dependence of the rate constants has also been investigated. Plots of $\log k_{1obs}$ and $\log k_{2obs}$ *versus* $1/T$ were always linear.

Product Analysis.—Dehydroreserpine is considered to be the first product of the consecutive reactions. The following evidences have been gathered to support this contention: (a) the wavelengths for excitation and fluorescence (obtained in a Perkin–Elmer 650–40 spectrofluorimeter) *viz.* 390 and 495 nm were of the natural fluoregen of dehydroreserpine,[5] (b) a thin layer chromatographic analysis revealed that the R_F value for the first product of the reaction mixture was identical to that of the product resulting from reserpine oxidation by nitrite,[4] and (c) this product showed a maximum at 383 nm which is characteristic of dehydroreserpine.[8]

At the end of the reaction, tetradehydroreserpine, a blue fluorescence product, was obtained. The wavelengths for

Table 1. Observed rate constants for the oxidation of reserpine by peroxodisulphate at 25 °C and 1N-H_2SO_4

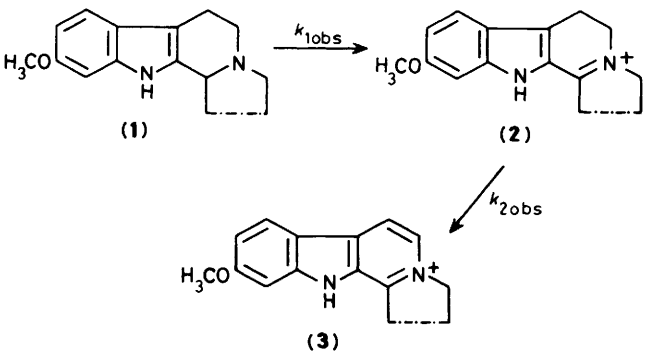
[Reserpine]/M	10^2 [$K_2S_2O_8$]/M	% v/v MeOH–water	$10^2 k_{1obs}$/min^{-1}	$10^2 k_{2obs}$/min^{-1}
10^{-4}	1	20	2.95	2.45
10^{-4}	2	20	5.44	4.46
10^{-4}	4	20	9.28	7.31
10^{-4}	6	20	12.35	9.39
10^{-4}	9	20	16.22	12.09
5×10^{-4}	9	30	9.88	8.34
10^{-4}	9	30	9.94	8.25
1.5×10^{-4}	9	30	10.00	8.20

Table 2. (a) Dependence of the observed rate constants on temperature. [reserpine] = 10^{-4}M, [$K_2S_2O_8$] 6 × 10^{-2}M, 1N-H_2SO_4, and 20% v/v MeOH–water

	Temperature (°C)					E_A/kJ
	20	25	30	35	40	mol^{-1}
$10^2 k_{1obs}$/min^{-1}	9.11	12.35	17.33	21.35	28.83	44 ± 5
$10^3 k_{2obs}$/min^{-1}	7.59	9.39	12.30	18.10	23.50	44 ± 9

(b) Peak potentials for anodic oxidation of reserpine, reserpiline, and yohimbine in lithium chloride (0.2M in MeOH)

	$E_{\frac{1}{2}}$/mV (*vs.* s.c.e.)
Reserpine	620, 800 (two waves)
Reserpiline	880
Yohimbine	no wave



Scheme 1.

excitation and fluorescence, *viz.* 330 and 435 nm, were the same as those for tetradehydroreserpine.[11]

Electrochemical Measurements.—A study has been carried out on the electrochemical oxidation behaviour of reserpine, yohimbine, and reserpiline by cyclic linear sweep voltammetry on a glassy carbon electrode. Lithium chloride (0.2M in methanol) has been used as supporting electrolyte. Voltammetric measurements have been performed as described in a previous communication.[12]

Results

The values of k_{1obs} and k_{2obs} (observed rate constants for the dehydroreserpine and tetradehydroreserpine formation respectively) are included in Table 1. Rate data in Table 1 show that no significant changes in the values of k_{1obs} and k_{2obs} occurred when the reserpine concentration is varied while keeping peroxodisulphate concentration in excess, which confirms the first-order dependence on the alkaloid. This behaviour has also been observed for other *Rauwolfia serpentina* alkaloids.[9]

The results of kinetic runs at different peroxodisulphate concentrations are also recorded in Table 1. As can be noted, an increase in the peroxodisulphate concentration produces an increase in the rate constant values. Plots of k_{1obs} and k_{2obs} *versus* the oxidant concentration are indeed curved. The same behaviour has been observed for other alkaloids.[9]

The results of kinetic runs at different temperatures are collected in Table 2, which also contains the activation energies, analysed using the least-squares procedure, together with their confidence limits. These standard deviations have been calculated with a 95% confidence level.

Finally, the results of the electrochemical measurements are also recorded in Table 2. According to these results, the sequence of anodic oxidation facility is: reserpine > reserpiline > yohimbine.

Discussion

A total reaction scheme for the oxidation of reserpine to tetradehydroreserpine is shown in Scheme 1 which indicates that the behaviour of reserpine, in the presence of a large excess of peroxodisulphate ions, is mathematically equivalent to consecutive first-order reactions with pseudo-first-order rate constants, k_{1obs} and k_{2obs}.

As it has been observed for other *Rauwolfia* alkaloids[9] and for the oxidation of 2,3-disubstituted indoles,[13] each of the aforementioned steps would involve the formation, *via* a reversible step, of an indolenine-type intermediate which would give the dehydro or the tetradehydro derivative, probably through intramolecular electron transfer (Scheme 2).

$$(1) + S_2O_8^{2-} \overset{K}{\rightleftharpoons} (I) \xrightarrow{k^1} (2)$$
$$(2) + S_2O_8^{2-} \overset{K'}{\rightleftharpoons} (I') \xrightarrow{k^2} (3)$$

Scheme 2.

According to this mechanism, the observed rate constant value for the formation of (2) would be[9] as in equation (2), and for the formation of (3), equation (3).

$$k_{1obs} = \frac{K k_1[S_2O_8^{2-}]}{1 + K[S_2O_8^{2-}]} \quad (2)$$

$$k_{2obs} = \frac{K' k_2[S_2O_8^{2-}]}{1 + K'[S_2O_8^{2-}]} \quad (3)$$

It is possible mathematically to manipulate equations (2) and (3) to yield expressions (4) and (5). These equations imply that if

$$\frac{1}{k_{1obs}} = \frac{1}{k_1 K[S_2O_8^{2-}]} + \frac{1}{k_1} \quad (4)$$

$$\frac{1}{k_{2obs}} = \frac{1}{k_2 K'[S_2O_8^{2-}]} + \frac{1}{k_2} \quad (5)$$

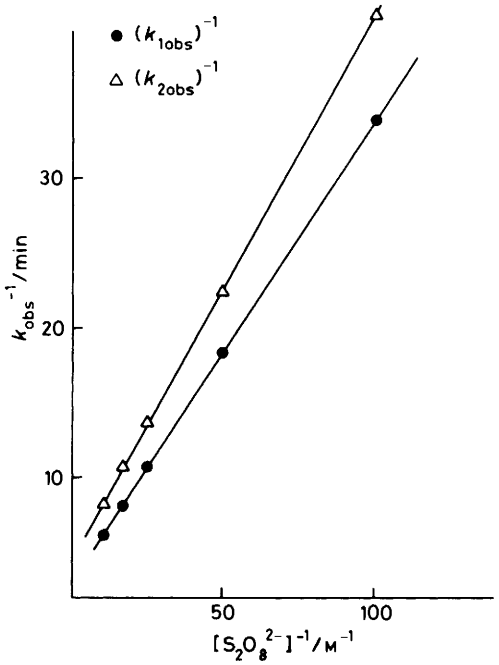


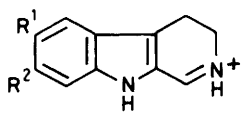
Figure 2. Plots of $(k_{1obs})^{-1}$/min (●) and 10^{-1} $(k_{2obs})^{-1}$/min (△) against $[S_2O_8^{2-}]^{-1}/M^{-1}$

the original assumptions are correct, the plots of the reciprocal of the rate constants *versus* the reciprocal of the peroxodisulphate concentration should yield straight lines. Figure 2 contains such a plot and it is indeed linear. Using the above plot, the following constant values can be computed: K 9.35 l mol^{-1}, k_1 0.345 min^{-1}, K' 12 l mol^{-1}, and k_2 2.26 10^{-2} min^{-1}.

At this point, it seems useful to analyse the nature of the indolenine intermediate. In the oxidations of various *Rauwolfia* alkaloids by peroxodisulphate,[9] the intermediate is formed by peroxodisulphate attack at C-3 of the indole ring, with displacement of a sulphate ion. It is reasonable to assume a similar mechanism for the oxidation of (**1**) instead of the radical mechanism proposed for other peroxodisulphate oxidations.[14,15] In fact, the activation energies support this contention, since the results in Table 2 clearly indicate that the rate-limiting step does not involve peroxodisulphate homolysis (E_A *ca.* 125 kJ mol^{-1}), but rather nucleophilic displacement on peroxide oxygen (E_A *ca.* 42 kJ mol^{-1}).[16]

Although the oxidation mechanism can be considered similar, there is an important difference between reserpine and *e.g.* reserpiline or yohimbine oxidation. As mentioned earlier, reserpine is first oxidized to the dehydro derivative (**2**) and this product is subsequently converted into the tetradehydro derivative (**3**). However, the final product of yohimbine oxidation is dehydroyohimbine while in the case of the reserpiline oxidation the tetradehydro derivative is obtained only when the reaction is continued for a long time.

As indicated above, the most important structural difference among these alkaloids is the position of the methoxy group in the indole nucleus. From our electrochemical data, it can be seen that reserpine (with a 6-methoxy group) is more easily oxidized than reserpiline (with 5,6-dimethoxy groups); yohimbine (without any methoxy group) does not show anodic oxidation in our experimental conditions. That is, the 6-methoxy group in the indole nucleus favours anodic alkaloid oxidation, while the methoxy group in position 5 and the absence of any methoxy group seem to hinder it. A direct



(**4**) $R^1 = OCH_3$, $R^2 = H$
(**5**) $R^1 = H$, $R^2 = OCH_3$
(**6**) $R^1 = R^2 = H$

comparison using kinetic data cannot be done since the kinetic runs were carried out under very different experimental conditions (*e.g.* ionic strength, dielectric constant, *etc.*). However, it seems reasonable to assume a similar reactivity pattern for the dehydro derivatives. Accordingly, dehydroreserpine should be easily oxidized, dehydroreserpiline should show less reactivity, and dehydroyohimbine should not react appreciably.

To gain a deeper insight into the influence of the methoxy position on the reactivity pattern of the dehydro derivatives, theoretical calculations have been carried out. Given the impossibility of calculating molecular parameters for the real dehydroderivatives, the simplified models (**4**)—(**6**) have been used. These models have been selected in order to retain the most important skeletal characteristics of our compounds.

Because of the number of parameters to be optimized the semiempirical CNDO/2[17] and INDO[18] methods have been chosen to maintain the computation time within reasonable limits. The GEOMO program in which the geometric optimization is carried out using the Rinaldi algorithm[19] has been utilized.

Since alkaloids are very large molecules, it seems reasonable to assume that orbital control is more important than charge control.[20] For this reason the reactivity is determined by the energy difference between the HOMO of the alkaloid (model) and the LUMO of the peroxodisulphate ion. When the methoxy group is in position 5, the HOMO energy is −0.0051 a.u. (CNDO/2) and −0.0156 a.u. (INDO); when it is in position 6, its energy is −0.0003 a.u. (CNDO/2) and −0.0065 a.u. (INDO); and if there is no methoxy group its energy is −0.0053 a.u. (CNDO/2) and −0.00156 (a.u. (INDO). The smaller the difference HOMO (alkaloid or model) − LUMO-(peroxodisulphate) the higher is the reactivity. According to this, the 6-methoxy group favours the oxidation process in excellent agreement with the experimental results.

Finally, as already mentioned, the product obtained using peroxodisulphate as oxidizing agent is different to that formed when sodium nitrite and vanadium pentaoxide are used as oxidizing agent. With the latter, only dehydroreserpine is observed and this fluorescence derivative seems to be stable.[3,7] However, Szalkowski[3] has found that its absorbance and stability decrease if the added sulphuric acid concentration is of a normality greater than one and when the amount of sodium nitrite exceeds 8 g l^{-1}. Also, suppression of the maximum fluorescence by higher concentrations of nitrous and sulphuric acid has been reported. This suppression has usually been referred to as a quenching effect. Nevertheless, this behaviour has also been observed when reserpine is oxidized with hydrogen peroxide or u.v. light, and in these cases the suppression fluorescence becomes more evident. In fact, when the reserpine solutions are irradiated with u.v. light for a long time, not only does the yellowish green fluorescence diminish, but also a blue fluorescence product arises. Ljumberg,[21] has assigned the name lumireserpine to this product which is in fact, identical to tetradehydroreserpine.[8]

These contradictory results could be explained by the nature of the oxidizing agent. As the present work shows, the oxidation of (**1**) is a consecutive reaction, its final product being tetradehydroreserpine (**3**). If a strong oxidant like peroxodisulphate is used, it is possible to observe the second step. With

weaker oxidant agents, this second step could be either thermodynamically or kinetically unfavourable under the experimental conditions used by the authors.[3,7] However, care should be excercised in interpreting the results obtained under different experimental conditions. In fact, the suppression of fluorescence claimed by several authors[7] could be due to the oxidation of dehydroserpine (**2**).

Acknowledgements

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