Mechanism of the oxidation of yohimbine and two of its 7*H*-substituted derivatives by sodium peroxodisulfate

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The mechanism of the oxidation of a Rauwolfia alkaloid, yohimbine, **Y**, by sodium peroxodisulfate, PDS, has been investigated in 40% v/v methanol–water at different PDS and acid concentrations. Because 3*H*-indoles have been reported as intermediates in the oxidation of this alkaloid, we have also analyzed the reactivity of 7-acetoxy- and 7-methoxy-7*H*-yohimbine, **AcOY** and **MeOY**, respectively. From these studies, we conclude that in the oxidation of **Y** with PDS, the first step of the reaction is an electrophilic attack of PDS at the C-7 atom of the substrate, to form the corresponding 3*H*-indole intermediate. This step is always the same, independent of the range of sulfuric acid concentration. Once the 3*H*-indole intermediate is formed, an imonium–enamonium equilibrium is established, the reactivity of the tautomeric forms and, therefore, the reaction products being strongly dependent on the media. That is, competing parallel reactions occur. In high sulfuric acid and low PDS concentrations, a rearrangement step, from the enamonium tautomer, produces the dehydroderivative, **DH**. At low acidities and high PDS concentrations, a new electrophilic PDS attack at the enamonium tautomer gives rise to the dioxy derivative, **Dx**. Under intermediate concentrations of PDS and acid where both steps compete, both reaction products are obtained. A global mechanism is postulated to account for the experimental rate equations.

Introduction

We have recently reported a mechanistic study of the oxidation reactions of 2,3-dimethylindole[1] and several 2,3-dialkylindole derivatives[2] by peroxodisulfate, PDS, and peroxomonosulfate, PMS, anions. This study, carried out mainly in sulfuric acid media, 0.1 M, afforded a detailed description of the steps in the mechanism of these reactions. Briefly, the reaction pathway always began with an electrophilic attack of the peroxidic bond at the indole ring to form the corresponding indole intermediates (see Chart 1). The subsequent steps of the reactions and,

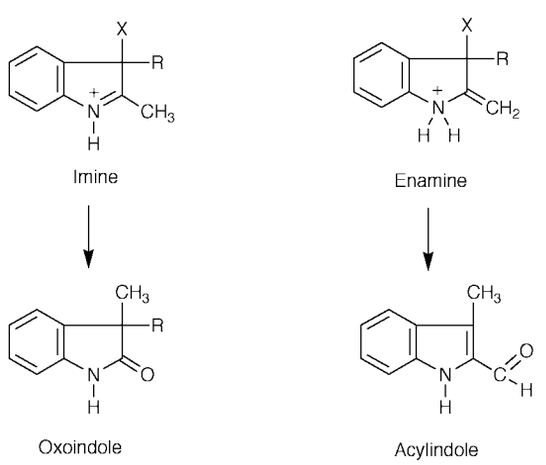

Chart 1

therefore, the reaction products depended upon the acidity of the media and the structures of the substrates. Thus, acylindole or oxoindole derivatives could be obtained as the final products.

As had been proposed by other authors,[3–5] and was concluded in our previous papers, the key step in the formation of acylindoles is the tautomerization of the indole intermediate to an exocyclic enamine. Once the enamine tautomer has been formed, it suffers a second peroxoanion attack to give the acyl derivative. However, the structure of the substrate and/or the acidity of the media[6] can hinder or block the enamine formation. In such a case, the imine tautomer or oxoindole derivatives are obtained as the final products. Thus, protonation of the indole intermediate favors the enamine formation in all the indoles studied, except for 2-phenyl-3-methylindole and tetrahydrocarbazole. For the first substrate, the presence of the phenyl group blocks the formation of the enamine and the reaction product is the imine tautomer. In the second case, the exocyclic six-membered ring hinders the stabilization of the planar enamine derivative. Thus, only under strong acidic conditions, some acyl derivative with other reaction products are obtained. In summary, the acidity of the media and the structural characteristics of the substrates must be considered to postulate the different steps in the mechanism of indole derivative oxidations.

Over the years, we have been interested in the physicochemical properties of several indole alkaloids of the Rauwolfia family. These alkaloids have long attracted interest on account of their biological activity,[7–9] some of them having therapeutic importance as sedative and antihypertensive agents. All of them possess, as the common unit, the tetrahydrobetacarboline ring. Therefore, as indole derivatives, many of their properties are similar to those of the simplest indoles. However, the presence of the piperidinic ring confers on them distinct characteristics.

Among their physicochemical properties, their oxidation reactions have special interest and they have been the objects of several papers.[10–15] Thus, it is well known that, depending on the reaction conditions, yohimbine, **Y**, can be oxidized to its fluorescent dehydro derivatives or to oxoindole compounds. It has also been reported that both reaction products come unquestionably from the rearrangement of an indole intermediate. Thus, according to Finch *et al.*,[12,13] on treatment with acetic or dilute mineral acid, the 7*H*-acyloxy derivatives of yohimbine and related alkaloids eliminated the acyloxy group and produced dehydro compounds, **DH**. However, when they were refluxed in methanol with a few drops of acetic acid the product isolated was the oxoindole, **Ox**. Also, Zinnes and Shavel[14] established that the 7*H*-chloroyohimbine was the

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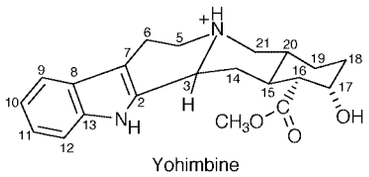
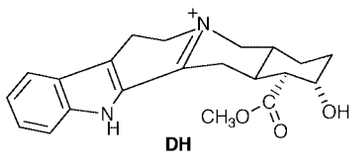
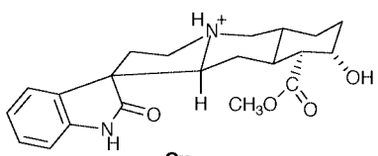


Yohimbine



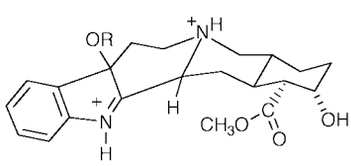
DH



Ox

common intermediate which could be converted to the dehydro-derivative or the oxoindole analog by employing the appropriate conditions. However, up to now there has been neither a mechanistic study on these reactions, nor has the influence of the media been cleared up. Moreover, because this alkaloid is an indole derivative, once the 7*H*-derivative has been formed one question arises: Could it behave as the 3*H*-derivatives of simplest indoles and, therefore, suffer a second electrophilic attack at the enamonium tautomer under the appropriate conditions? This would mean that apart from the formation of the **DH** and oxoindole derivatives, a different product should be obtained.

Thus, in the light of the results obtained in the oxidation reactions of simplest indoles, we feel that to build up a detailed mechanistic picture of the oxidation reactions of indole alkaloids, further studies are needed. Therefore, we have decided to revisit them. With this purpose, we selected sodium peroxodisulfate as the oxidizing agent. This compound is well known as an excellent and versatile oxidant for a variety of organic compounds including indole derivatives.[10,11,16] As the substrate, the Rauwolfia alkaloid, yohimbine, **Y**, was chosen. Also, two indole derivatives, 7*H*-acetoxy and 7*H*-methoxyyohimbines, **AcOY** and **MeOY** respectively, have been synthesized.[12,17] We have supposed these indoles to be adequate model compounds



7*H*-Yohimbines

AcOY: R = $COCH_3$
MeOY: R = CH_3

for the intermediate in the oxidation of **Y** with PDS. Thus, the study of their reactivities against PDS should help to understand the mechanism of **Y** oxidation. To ascertain the influence of the media in the steps of the mechanism, the work has been carried out under two extreme conditions of acid and PDS concentrations. Also, the reactivity of one of the derivatives, **AcOY**, has been studied under intermediate conditions of acidity and PDS concentrations. All these results allow us to establish the experimental rate equations and help to confirm the generality of the postulated mechanism.

Experimental

Reagents

Yohimbine, **Y**, (Aldrich Química) was used as received. Its derivatives were synthesized according to the methods described in the bibliography.[12,17] Stock solutions of the substrates were prepared in methanol, stored in the dark and frequently renewed. Sodium peroxodisulfate, PDS, was AnalaR grade (Merck). All the solutions had a final proportion of 40% v/v methanol–water. The acidity of the media was adjusted by the addition of the appropriate amounts of standardized sulfuric acid solutions. Over the whole range of acid concentration used in this work, the substrates are protonated. Ionization data obtained spectrophotometrically by the usual procedure gave apparent pK_a values, in 40% v/v methanol–water, of 6.14 ± 0.02 and 5.85 ± 0.02 for **AcOY** and **MeOY**, respectively.

To ensure the formation of a sole reaction product, two extreme experimental conditions were used: high sulfuric acid, (0.7–1.2) mol dm^{-3}, and low PDS, (2–10) × 10^{-3} mol dm^{-3}, concentrations, and low sulfuric acid, (0.03–0.15) mol dm^{-3}, and high PDS, (4–16) × 10^{-2} mol dm^{-3}, concentrations. Under these conditions, clean isosbestic points always appear in the spectra of the reaction mixtures. Because ions can be involved in the reactions, the ionic strength, I, was kept constant by the addition of $NaClO_4$. Thus, kinetic measurements were carried out at I = 4 and 0.65 mol dm^{-3} in media of high and low acid concentrations, respectively. In the range of intermediate conditions, $HClO_4$ acid was used to avoid possible changes in the concentration of HSO_4^- anions. As we will see later, the concentration of these species can participate in the experimental rate law equation. Therefore, it was kept constant, 0.12 mol dm^{-3}, by the addition of $NaHSO_4$ salt and the acidity of the media was changed with $HClO_4$ acid. Also, an intermediate ionic strength, I = 2 mol dm^{-3}, was maintained with $NaClO_4$.

Finally, as it is known, experimental conditions of high acid concentrations and ionic strength are far from being covered by simple thermodynamic approaches. Therefore, the kinetic results should be interpreted using proton activities rather than proton concentrations. Unfortunately, experimental data on hydrogen ion activities in 40% v/v methanol–water solutions at different acid concentrations and ionic strengths are not available in the literature. Our solution to this problem was to check the results obtained using proton activities in water [18,19] or directly proton concentrations. Because the resulting kinetic laws were independent on the approach used, we decided, for the sake of simplicity, to use proton concentrations instead of proton activities.

Kinetic measurements

The evolution of the absorption spectra of the mixtures with time was recorded with a computer-interfaced Hewlett-Packard 8452A diode array spectrophotometer. Kinetic measurements were done under pseudo-first-order conditions with a large excess of PDS. In media of high sulfuric acid concentrations we monitored both the disappearance of the substrate at 278 nm (**Y**) or 290 nm (**AcOY** and **MeOY**) and the appearance of the reaction product at 354 nm. In media of low sulfuric acid concentrations, the reactions were followed at 278 or 290 nm. The temperature was always maintained at 298.1 ± 0.1 K. Pseudo-first-order rate constants were obtained by a nonlinear least-square fitting of the absorbance–time data to eqn. (1):

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

Usually, an excellent agreement between experimental and calculated A_t values was obtained. Rate constants in duplicate runs were reproducible to within 5%.

The possibility that the hydrolysis of PDS to peroxomonosulfate could compete with the oxidation of the substrates

was tested by independent measurements. These experiments showed that, under our acidity conditions the hydrolytic reactions are much slower than the oxidation reactions. Finally, we have checked that the presence of radical promoters or radical traps did not affect the oxidation rates. This excludes the involvement of radical PDS species on the reactions.

Results

Characterization of the reaction products

In media of high sulfuric acid concentrations, TLC experiments showed the formation of a sole reaction product. This product was unambiguously characterized as the dehydroderivative, **DH**[20] (Chart 2), by comparison of its spectral properties with those of a pure sample prepared by an independent procedure.

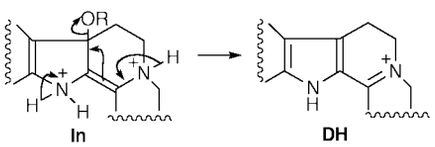


Chart 2

At low sulfuric acid concentrations, TLC experiments (benzene–ethyl acetate–diethylamine, 7 : 2 : 1) showed that, under the experimental conditions used for kinetic measurements, a unique reaction product was obtained. Its separation and characterization was made as follows: **Y**, 300 mg, was stirred in a solution containing sulfuric acid, 0.4 M, PDS in the solubility limit and 40% v/v methanol–water. After several hours, the solution was made slightly basic, pH ~9, with sodium hydroxide and extracted with three portions of dried ethyl acetate. Removal of the solvent under reduced pressure, gave a yellow solid which was passed through alumina and eluated with benzene–ethyl acetate–diethylamine, 7 : 2 : 1. From the eluate, 30 mg of yellow needlelike crystals were obtained, UV λ_{max} in MeOH 214, 254 nm. This solid was a mixture of two compounds independent of whether **Y**, **AcOY** or **MeOY** were used as the reacting substrates. Thus, TLC experiments showed that, in addition to the spot corresponding to the kinetic reaction product, a new spot at higher R_f, which was split into two near overlapping stains, also appears. This second product, was easily identified as the oxoindole derivative, **Ox**, being present in the mixture in its two stereoisomeric forms. As mentioned before, **Ox** is an usual oxidation product of **Y** in media of low acidity. Probably, due to the much larger period of time needed for the preparative experiments as compared with the kinetic experiments, the formation of the **Ox** derivative cannot be avoided. The identification of the **Ox** derivative was made by TLC comparison with an independently synthesized sample of this compound. The mass spectrum of the pure sample of the **Ox** derivative, MS(FAB): *m*/*z* (%): 371(100) [$M^+ - H$], and the comparison of its ^{13}C NMR shifts in MeOD with published data for related compounds[21] confirmed this product to be the **Ox** derivative. Also, its NMR spectrum shows the presence of the two stereoisomers of this compound in solution. Thus, carbon signals were double, being the chemical shifts of the most representative carbons: δ: 181.7, 182.4 (C2), 72.4, 74.2 (C3), 53.8, 53.9 (C5), 34.2, 35.2 (C6), 56.0, 56.9 (C7), 133.0, 133.7 (C8) and 141.4, 141.9 (C13).

The identification of the reaction product, that with lower R_f, was made in an indirect way. Unfortunately, as already mentioned, we have been unable to obtain it in its pure form and, therefore, we have tried to elucidate its chemical structure from the differences between the spectra of the mixture and that of the pure sample of the **Ox** derivative. As would be expected, the ^{13}C NMR spectrum of the mixture is complicated by the simultaneous presence of the unknown compound and the two stereoisomers of the **Ox** derivative. However, a new signal, δ_C(100 MHz; CD_3OD) 84 ppm, non-existent either in the spectrum of the **Ox** compound or in the spectrum of **Y**,[22] and corresponding to a tertiary carbon atom clearly appears in the spectrum of the mixture. Such a chemical shift has previously been reported for the C-3 atom in a compound of structure similar to that of the **Dx** compound (Chart 3). Specifically, a

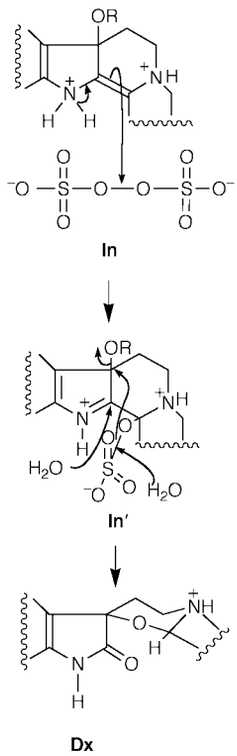


Chart 3

chemical shift of 87.04 ppm has been published for this carbon atom in dioxyreserpine,[23] one of the oxidized forms of reserpine which is an alkaloid also belonging to the Rauwolfia family. It is worth mentioning that such a signal is neither observed in the NMR spectra of **AcOY** and **MeOY** nor in the spectrum of the 7*H*-hydroperoxyreserpine.[23] Moreover, the mass spectrum of the mixture shows: MS(FAB): *m*/*z* (%): 387(100) [$M^+ - H$] 371(30). That is, apart from the signal corresponding to the **Ox** derivative, the signal shifted sixteen units also points to the presence of an extra oxygen atom in the molecule. These evidences allow us to suggest a structure of type **Dx** (Chart 3) for our kinetic reaction product. As we will see in the Results section, kinetic evidence supports the formation of such a compound. Thus, the fact that in these media the reactivity of the 7*H*-derivatives depends on PDS concentration, implies a peroxo attack at the substrate (Chart 3). Thus, the reaction product is necessarily an oxidized form of the **Ox** derivative.

It is worth noting that, as one of the refereees has suggested, the OR group displacement involved in this reaction could be a stereochemically controlled process. Thus, as in related reactions,[20] the rearrangement of **In′** species would presumably only occur if the sulfate group is *anti* to the OR group. The other stereoisomer should not rearrange and could probably account for the existence, as we will see later, of the reversible step postulated for PDS attack.

Kinetic results

As is shown typically in Fig. 1, the evolution of the reaction mixtures with time strongly depends on the range of sulfuric acid and PDS concentrations. Thus, at high acidities, Fig. 1a, upon mixing the reactants there is a decrease in the absorbance

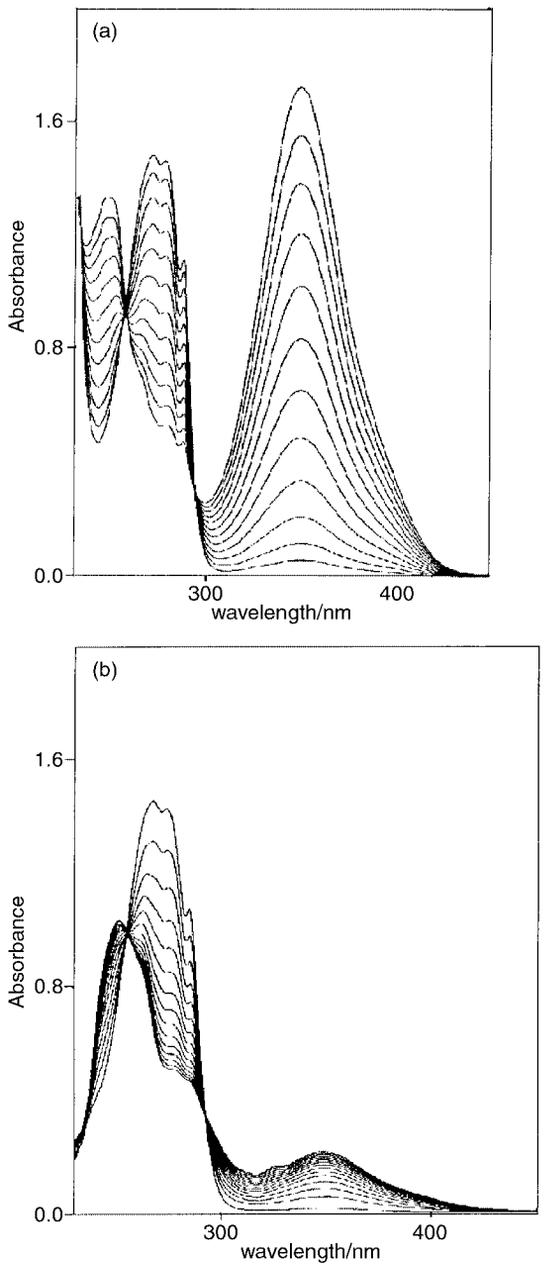


Fig. 1 Changes in the UV-vis spectra of **Y** for a typical reaction mixture in 40% v/v methanol–water: [**Y**] = 2×10^{-4} mol dm^{-3}, (a) [PDS] = 5×10^{-3} mol dm^{-3} and [H_2SO_4] = 1 mol dm^{-3}, I = 4 mol dm^{-3}, (b) [PDS] = 5×10^{-2} mol dm^{-3} and [H_2SO_4] = 0.05 mol dm^{-3}, I = 0.65 mol dm^{-3}. Δt = 10 min.

at the maximum wavelength of the substrate. Simultaneously, a new absorption band appears at 354 nm. This band is characteristic of the partially aromatized compounds, the dehydro-derivatives, **DH**.[20] At low sulfuric acid concentrations, Fig. 1b, the disappearance of the 278 nm band is accompanied by the growing of a band around 250 nm that is typical of an oxoindole chromophore. Also, a very small increase of the absorbance at 354 nm is observed.

Upon mixing the indole intermediates with PDS, the spectra change in a completely similar way. Thus, the band of the protonated substrates (p$K_a \sim 6$), at 290 nm, disappears and, depending on the acidity of the media, bands of the dehydro or oxoindole derivatives are observed. The clean isosbestic points in all the spectra show the direct transformation of the substrates into the reaction products. Always, the measured rate constants at fixed PDS and acid concentrations were the same over a range of substrate concentrations. This agrees with a first-order dependence on the latter reactant concentration.

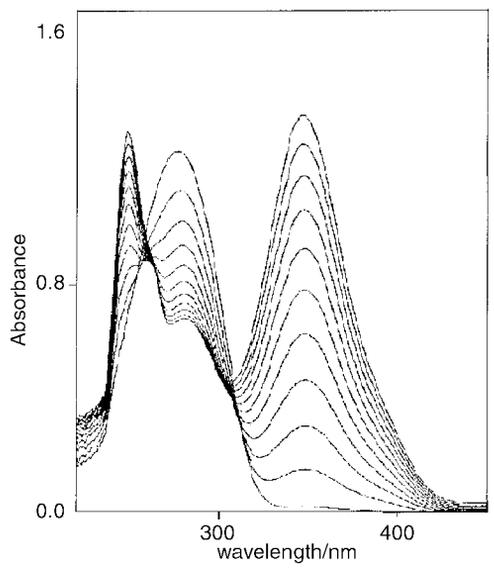


Fig. 2 Changes in the UV-vis spectra of **AcOY** in the intermediate range of acidity in 40% v/v methanol–water: [**AcOY**] = 2×10^{-4} mol dm^{-3}, [$HClO_4$] = 0.2 mol dm^{-3}, [HSO_4^-] = 0.12 mol dm^{-3} and [PDS] = 7×10^{-2} mol dm^{-3}, I = 2 mol dm^{-3}.

In media of high sulfuric acid concentrations, the rate constants of the reaction between **Y** and PDS, at fixed [H^+] = 0.8 mol dm^{-3}, vary linearly with [PDS] with slope $(3.6 \pm 0.4) \times 10^{-2}$ dm^3 mol^{-1} s^{-1} and zero intercept at the origin. Also, the plot of the rate constants, at fixed [PDS] = 6×10^{-3} mol dm^{-3}, against proton concentrations, is a straight line passing through the origin with slope of $(4 \pm 1) \times 10^{-4}$ dm^3 mol^{-1} s^{-1}. The ionic strength of the media did not affect the values of these rate constants. In the case of the 7*H*-derivatives, the rate constants are independent of PDS concentrations, but they depend linearly on proton concentrations with slopes $(2.3 \pm 0.6) \times 10^{-4}$ and $(9.1 \pm 0.6) \times 10^{-4}$ dm^3 mol^{-1} s^{-1} for **AcOY** and **MeOY**, respectively. The intercept at the origin is zero for **MeOY** and $(3.1 \pm 0.6) \times 10^{-4}$ s^{-1} for **AcOY**. Also, these rate constants increase on increasing the ionic strength.

In the range of low sulfuric acid concentrations, the rate constants for the three substrates, at fixed [H^+] = 0.05 mol dm^{-3}, vary linearly with [PDS]. The slopes of these plots, which have zero intercept at the origin, are $(4.7 \pm 0.4) \times 10^{-3}$, $(5.8 \pm 0.9) \times 10^{-3}$ and $(4.0 \pm 0.5) \times 10^{-3}$ dm^3 mol^{-1} s^{-1} for **Y**, **AcOY** and **MeOY**, respectively. Also, the plots of the rate constants, at fixed [PDS] = 6×10^{-2} mol dm^{-3}, for **Y** and **MeOY** and [PDS] = 4×10^{-2} mol dm^{-3} for **AcOY**, against [H^+] are linear with zero intercept at the origin. The slopes of these plots are $(3.5 \pm 0.8) \times 10^{-3}$, $(3.3 \pm 0.7) \times 10^{-3}$ and $(6.7 \pm 0.3) \times 10^{-3}$ dm^3 mol^{-1} s^{-1} for **Y**, **AcOY** and **MeOY**, respectively. Under these conditions, an increase of the ionic strength produces a decrease of the rate constants for all the substrates.

Finally, as already mentioned, we have also carried out kinetic measurements for the oxidation of **AcOY** with PDS in an intermediate range of acidity, PDS concentrations and ionic strength. These experiments were done at fixed HSO_4^- concentration and varying both $HClO_4$ and PDS concentrations. As Fig. 2 shows, under these conditions, both reaction products are obtained and no clear isosbestic points appear. The observed rate constants are shown in Table 1. From the data in this Table, the following facts should be emphasized: Firstly, the rate constants at fixed [H^+] vary linearly with PDS concentrations. The slopes of these plots, m_1, increase with [H^+], but the intercepts at the origin, b_1, are independent of this parameter. Secondly, at constant PDS concentrations, the plots of the rate constants against [H^+] are also linear. In this case, both, the slopes of the plots, m_2, and the intercepts at the origin, b_2, increase on

Table 1 Observed rate constants in the intermediate range of acidity at different PDS and $HClO_4$ concentrations. [**AcOY**] = 2.0×10^{-4} mol dm^{-3}, [HSO_4^-] = 0.12 mol dm^{-3}, I = 2 mol dm^{-3}, 40% v/v methanol–water and 298.1 K

	$k_{obs}/10^{-4}$ s^{-1}				
	[$HClO_4$]/mol dm^{-3}				
[PDS]/10^{-2} mol dm^{-3}	0.1	0.2	0.3	0.35	0.4
5.2	2.97	3.41	4.08	4.44	4.78
7.0	3.54	4.22	4.91	5.62	6.00
10.0	4.32	5.30	6.12	7.04	7.58
12.0	4.92	6.08	7.18	8.17	8.93

Table 2 Values of the slopes, m_1, of k_{obs} *vs*. PDS concentration plots at different proton concentrations[a]

[$HClO_4$]/mol dm^{-3}	$m_1/10^{-3}$ dm^3 mol^{-1} s^{-1}
0.1	2.8 ± 0.2
0.2	3.9 ± 0.4
0.3	4.5 ± 0.5
0.35	5.3 ± 0.7
0.4	5.9 ± 0.7

[a] The intercepts at the origin, b_1, are independent of [H^+]. Average value of $b_1 = (1.6 \pm 0.4) \times 10^{-4}$ s^{-1}.

Table 3 Values of the slopes, m_2, and the intercepts at the origin, b_2, of k_{obs} *vs*. [$HClO_4$] plots at different PDS concentrations

[PDS]/10^{-2} mol dm^{-3}	$m_2/10^{-4}$ dm^3 mol^{-1} s^{-1}	$b_2/10^{-4}$ s^{-1}
5.2	6.2 ± 0.9	2.3 ± 0.3
7.0	8.3 ± 1	2.6 ± 0.4
10.0	11.0 ± 0.8	3.2 ± 0.6
12.0	13.4 ± 1	3.5 ± 0.7

increasing PDS concentrations. These results are summarized in Tables 2 and 3. Also, Figs. 3 and 4 show the linearity of the plots of m_1 *versus* [H^+] and m_2 and b_2 *versus* PDS concentrations, respectively. From the plot of m_1 *versus* [H^+] a slope of $(10 \pm 2) \times 10^{-3}$ dm^6 mol^{-2} s^{-1} and an intercept of $(1.8 \pm 0.6) \times 10^{-3}$ dm^3 mol^{-1} s^{-1} are obtained. The slope of the m_2 *versus* PDS concentration plot is $(10.5 \pm 0.1) \times 10^{-3}$ dm^6 mol^{-2} s^{-1}. In this case, the intercept at the origin is zero. Finally, from the plot of b_2 against PDS concentration we can calculate a slope and an intercept at the origin of $(1.8 \pm 0.2) \times 10^{-3}$ dm^3 mol^{-1} s^{-1} and $(1.4 \pm 0.1) \times 10^{-4}$ s^{-1}, respectively.

Discussion

Because, presumably, a 7*H*-derivative is the intermediate in the oxidation of **Y** with PDS, we will start the discussion analyzing the reactivity of the model compounds, **AcOY** and **MeOY** with PDS. The results obtained in the present paper indicate that their reactivities strongly depend on the media. In media of high acid concentrations, where the **DH** compound is obtained, the observed rate constants do not depend on PDS concentration, but they increase linearly with the increase of acidity. These rate constants also increase with the increase of the ionic strength.

On the contrary, in the low acidity range the observed rate constants vary linearly with both PDS and H^+ concentrations. Furthermore, these rate constants decrease on increasing the ionic strength, indicating that ions of different charge sign could be involved. Unfortunately, pK_a values for PDS species in 40% methanol–water solutions have not been published. Furthermore, even in water, it has been reported[16] that the relevant pK_a values of peroxodisulfuric acid are not known

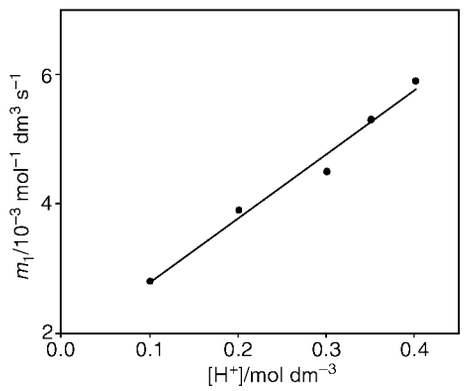


Fig. 3 Plot of the m_1 values, see Table 2, against [H^+] in the intermediate range of acidity.

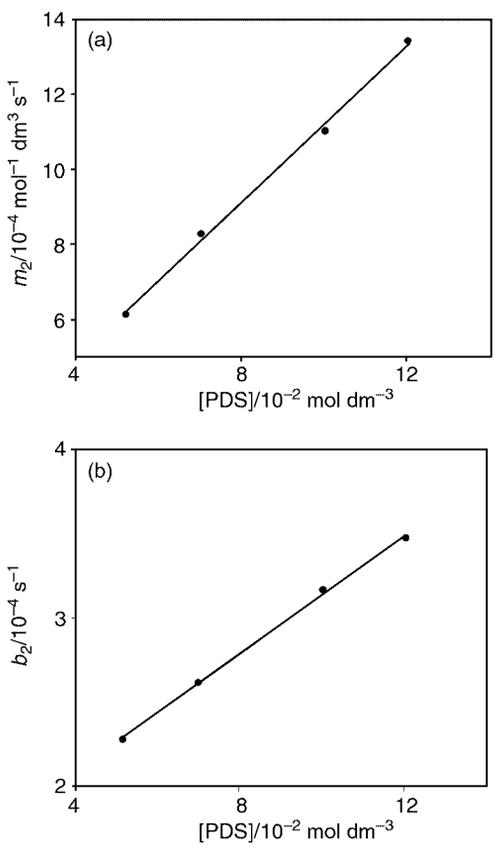


Fig. 4 Plot of (a) m_2 values and (b) b_2 values, see Table 3, against [PDS] in the intermediate range of acidity.

with any certainty. Therefore, we postulate that under our experimental conditions of low proton concentrations, $S_2O_8^{2-}$ anions are the electrophilic agents. After the electrophilic attack, an acid catalyzed hydrolysis of the leaving group to give the **Dx** compound is postulated.

Therefore, in the light of these results the most probable mechanistic schemes for these reactions are those shown in Scheme 1, pathway A or B for the reactions in high and low acid concentrations, respectively. **In** stands for the indole intermediate. The rate law for the mechanism in pathway A is shown in eqn. (2):

$$k_{obs} = k_0 + k_1 [H^+] \quad (2)$$

According to this equation, k_{obs} values should, as observed, vary linearly with [H^+]. The parameters obtained from the plots of k_{obs} against [H^+] allow us to calculate the following values for the constants in Scheme 1, pathway A: $k_1 = (2.3 \pm 0.6) \times 10^{-4}$

Pathway A

$$\mathbf{In} \xrightarrow{k_0} \mathbf{DH}$$

$$\mathbf{In} + H^+ \xrightarrow{k_1} \mathbf{DH}$$

Pathway B

$$HSO_4^- \overset{K_S}{\rightleftharpoons} H^+ + SO_4^{2-}$$

$$\mathbf{In} + S_2O_8^{2-} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathbf{In}' + SO_4^{2-}$$

$$\mathbf{In}' + H^+ \xrightarrow{k_4} \mathbf{Dx}$$

Scheme 1

and $(9.1 \pm 0.6) \times 10^{-4}$ dm^3 mol^{-1} s^{-1} for **AcOY** and **MeOY**, respectively. Also, for **AcOY** we can obtain a value for k_0 equal to $(3.1 \pm 0.6) \times 10^{-4}$ s^{-1}.

The rate law for the mechanism in pathway B with application of the equilibrium condition is given in eqn. (3):

$$k_{obs} = \frac{k_2 k_4 (S_2O_8^{2-})[H^+]}{k_{-2}(SO_4^{2-})} \quad (3)$$

Taking into account that a value around 2 has been reported for the pK_s in water of the pair HSO_4^-/SO_4^{2-},[24] we must assume that under our experimental conditions, the protonation equilibrium of SO_4^{2-} anions is established. Thus, using the stoichiometric relation, $[H_2SO_4]_0 = [HSO_4^-] + [SO_4^{2-}]$, the actual sulfate anion concentration should be expressed as $[SO_4^{2-}] = K_s[H_2SO_4]_0/([H^+] + K_s)$. Assuming that in these media of low acidities, $K_s \ll [H^+]$ and $[H_2SO_4]_0 \sim [H^+]$, eqn. (3) yields eqn. (4):

$$k_{obs} = \frac{k_2 k_4 (S_2O_8^{2-})[H^+]}{k_{-2}\, K_s} \quad (4)$$

According to this equation, k_{obs} exhibits, as observed, a linear dependence on proton and PDS concentrations with zero intercepts at the origin. The slopes of these plots allow us to calculate a mean value for the ratio $k_2k_4/k_{-2}K_s$ of $(10 \pm 1) \times 10^{-2}$ and $(9.6 \pm 0.7) \times 10^{-2}$ dm^6 mol^{-2} s^{-1} for **AcOY** and **MeOY**, respectively.

According to these results, if the indole intermediate can react through competing parallel reactions, it should be possible to find experimental conditions where the specific reaction rates were comparable and, therefore both reaction products obtained simultaneously (see Fig. 2). With this in mind, we have carried out kinetic measurements for **AcOY** in an intermediate range of acidity, PDS concentrations and ionic strength (see Table 1). Under these acidity conditions, we assume that, as it is shown in Scheme 2, both, $S_2O_8^{2-}$ and $HS_2O_8^-$ species can act

$$\mathbf{In} \xrightarrow{k_0'} \mathbf{DH}$$

$$\mathbf{In} + H^+ \xrightarrow{k_1'} \mathbf{DH}$$

$$HS_2O_8^- \overset{K_{a2}}{\rightleftharpoons} H^+ + S_2O_8^{2-}$$

$$\mathbf{In} + S_2O_8^{2-} \xrightarrow{k_2'} \mathbf{In}' + SO_4^{2-}$$

$$\mathbf{In} + HS_2O_8^- \underset{k'_{-3}}{\overset{k_3'}{\rightleftharpoons}} \mathbf{In}' + HSO_4^-$$

$$\mathbf{In}' + H^+ \xrightarrow{k_4'} \mathbf{Dx}$$

Scheme 2

as electrophilic agents.[16] Also, it is assumed that the leaving SO_4^{2-} group in step 2 immediately protonates to give HSO_4^- anions, which are the existing species at these acid concentrations. On the basis of this assumption, and according to the mechanism postulated in Scheme 2, it is expected that HSO_4^- anion concentration appears in the rate law equation. Therefore, its concentration has been kept constant by the addition of $NaHSO_4$ salt, 0.12 M, and the acidity of the media has been varied with $HClO_4$ acid. The rate law for the global mechanism in Scheme 2, with the application of the steady-state approximation to **In**′ and considering that $[PDS]_0 = [S_2O_8^{2-}] + [HS_2O_8^-]$, is shown in eqn. (5), where K_{a2} is the acidity constant for the $HS_2O_8^-/S_2O_8^{2-}$ equilibrium. According to this equation and assuming that under our experimental conditions $K_{a2} \gg [H^+]$ and $k_4'[H^+] \gg k'_{-3}[HSO_4^-]$, eqn. (6) should hold:

$$k_{obs} = k_0' + k_1'[H^+] + \left[\frac{k_2'K_{a2} + k_3'[H^+]}{K_{a2} + [H^+]}\right] \frac{k_4'[H^+][PDS]_0}{k'_{-3}[HSO_4^-] + k_4'[H^+]} \quad (5)$$

$$k_{obs} = k_0' + k_1'[H^+] + \left(k_2' + \frac{k_3'[H^+]}{K_{a2}}\right)[PDS]_0 \quad (6)$$

Hence, at fixed $[H^+]$, the rate constants should, as observed, vary linearly with $[PDS]_0$. Also, the slopes, $m_1 = k_2' + k_3'[H^+]/K_{a2}$, and the intercepts, $b_1 = k_0' + k_1'[H^+]$, of these plots *versus* $[H^+]$ should be linear. As Fig. 3 shows, the plot of m_1 values *versus* $[H^+]$ is, in fact, linear. However, as data in Table 2 show, the values of the intercepts do not change with $[H^+]$. This means that k_1' is very small, ~0. The other parameters obtained from these plots, allow us to calculate the following mean constants, $k_3'/K_{a2} = (10 \pm 2) \times 10^{-3}$ dm^6 mol^{-2} s^{-1}, $k_2' = (1.8 \pm 0.6) \times 10^{-3}$ dm^3 mol^{-1} s^{-1} and $k_0' = (1.6 \pm 0.4) \times 10^{-4}$ s^{-1}.

On the other hand, and also according to eqn. (6), at constant PDS concentration, k_{obs} values should, as observed, depend linearly with $[H^+]$ with slope, m_2, equal to $k_1' + k_3'[PDS]_0/K_{a2}$ and intercept at the origin, b_2, equal to $k_0' + k_2'[PDS]_0$. Moreover, these slopes and intercepts should exhibit a linear dependence with $[PDS]_0$. The linearity of these plots is shown in Fig. 4 (a) and (b). The parameters obtained from these plots allow us to calculate the following mean constants: $k_3'/K_{a2} = (10.5 \pm 0.1) \times 10^{-3}$ dm^6 mol^{-2} s^{-1}, $k_2' = (1.8 \pm 0.2) \times 10^{-3}$ dm^3 mol^{-1} s^{-1}, $k_0' = (1.4 \pm 0.1) \times 10^{-4}$ s^{-1}, and k_1' ~0. These values are in excellent agreement with those obtained previously. Moreover, on the basis of our assumption, $K_{a2} \gg [H^+]$, k_3' is at least five times greater than k_2'. This is of the right order of magnitude taking into account the better electrophilic character of $HS_2O_8^-$ anions.

At this point, it is interesting to mention some results obtained from additional kinetic experiments, also carried out in this intermediate range, and related with the participation of $[HSO_4^-]$ in the rate law equation. Thus, kinetic measurements performed in $HClO_4$ acid, 0.05 to 0.4 mol dm^{-3} and $I = 2$ mol dm^{-3} ($NaClO_4$), in the absence of HSO_4^- anions, have shown that the observed rate constants are independent of PDS concentrations, but they vary linearly with proton concentrations. Thus, on the basis of the mechanism in Scheme 2, we must assume that, in the absence of HSO_4^- anions, the backward step, k'_{-3}, no longer occurs, being the hydrolysis of the intermediate **In**′ the rate determining step. From the plot of the observed rate constants against $[H^+]$, values of $(1.44 \pm 0.06) \times 10^{-3}$ dm^3 mol^{-1} s^{-1} and $(1.57 \pm 0.01) \times 10^{-4}$ s^{-1} are obtained for the slope, k_4', and the intercept at the origin, k_0', respectively. Again, there is an excellent correspondence between this value for k_0' and those previously obtained.

Assuming that **AcOY** and **MeOY** are adequate model compounds for the indole intermediate in the oxidation of **Y** with PDS, we can broach the mechanism for the oxidation of this last substrate. The main difference in the kinetic behavior is observed in the high acid concentration range. Thus, in this range the observed rate constants depend linearly on PDS and proton concentrations. Both plots have zero intercept at the origin. As we have already checked, under these acidity conditions the reactivity of the 7*H*-derivatives is independent of PDS concentration. Thus, we conclude that for **Y** the rate

determining step is the electrophilic attack of PDS at the C-7 atom of the indole ring to form the 7*H*-sulfate intermediate, **Is** in Scheme 3. The dependence of the observed rate constants

$$H_2S_2O_8 \overset{K_{a1}}{\rightleftharpoons} H^+ + HS_2O_8^-$$

$$\mathbf{Y} + H_2S_2O_8 \xrightarrow{k_5} \mathbf{HIs} + HSO_4^-$$

$$\mathbf{Y} + HS_2O_8^- \xrightarrow{k_6} \mathbf{Is} + HSO_4^-$$

$$\mathbf{Is/HIs} \longrightarrow \mathbf{DH} \text{ fast}$$

Scheme 3

with $[H^+]$ can be ascribed to the protonation of $HS_2O_8^-$ species. To confirm this assumption, the reactivity of 2-phenyl-3-methylindole with PDS has been studied in this acidity range. This substrate was mainly selected for the following two reasons. Firstly, the observed rate constants correspond to the PDS attack at the indole ring to form the indole intermediate as the final product.[2] Secondly, because this substrate cannot be protonated in these media,[25] any influence of the acidity in the rate constants can only be attributable to the oxidant. As expected, the observed rate constants for this system vary linearly with PDS and proton concentrations with zero intercept at the origin. Therefore, according to the mechanism in Scheme 3, and considering that $[PDS]_0 = [HS_2O_8^-] + [H_2S_2O_8]$, the rate law in eqn. (7) should hold:

$$k_{obs} = \frac{k_5[H^+] + k_6K_{a1}}{K_{a1} + [H^+]}[PDS]_0 \qquad (7)$$

Because the rate constants for the reaction between **Y** and PDS do not depend on the ionic strength, we assume that neutral $H_2S_2O_8$ species, that are much better electrophiles than $HS_2O_8^-$ molecules, are the species attacking the C-7 atom of the substrate, *i.e.* $k_6K_{a1} \ll k_5[H^+]$. On this basis, and considering that $K_{a1} \gg [H^+]$, k_{obs} values should, as observed, depend linearly on $[PDS]_0$ and $[H^+]$ with zero intercept at the origin. The parameters obtained from these plots allow us to calculate a mean value of $(6 \pm 1) \times 10^{-2}$ dm^6 mol^{-2} s^{-1} for the k_5/K_{a1} relation.

In the low acidity range, the kinetic behavior observed in the oxidation of **Y** with PDS is completely similar to that observed for the indole intermediates. Thus, the rate constants vary linearly with PDS and H^+ concentrations with zero intercept at the origin. The similarity of the slopes of these plots with those measured for **AcOY** and **MeOY** suggests that, in this media we are also measuring the electrophilic attack of $S_2O_8^{2-}$ anions at the indole intermediate. That is, pathway B in Scheme 1 also operates for this substrate. Therefore, we conclude that the first electrophilic attack at the C-7 atom of **Y** is fast, the second PDS attack being the rate determining step. From the slopes of these plots, a mean value of $(8.0 \pm 0.9) \times 10^{-2}$ dm^6 mol^{-2} s^{-1} for the relation $k_2k_4/k_{-2}K_s$ can be obtained. This value is, as expected, similar to those obtained for **AcOY** and **MeOY**.

We can now answer the question we raised in the Introduction. Thus, **Y** can be oxidized with PDS to its **DH** derivative which is a particular product of these indole alkaloids. This product comes from a rearrangement of the indole intermediate assisted by the piperidinic nitrogen atom. However, it can also be oxidized to the **Dx** derivative as a result of a second PDS attack at the indole intermediate. With hindsight, the formation of such an unusual product is not surprising because the well-known oxidizing properties of PDS and the indolic structure of the substrate. In fact, as we have already mentioned, a recent report on the oxidation of a similar alkaloid, reserpine, characterizes dioxyreserpine as the major product. Thus, as expected, the indolic alkaloids can behave as the simplest indoles under the appropriate conditions. Interestingly, the results obtained in the present paper show that, in spite of the fact that $H_2S_2O_8$ species are much better electrophiles than $HS_2O_8^-$ molecules, **Y** only suffers the second peroxo attack in the low and intermediate acidity media. Therefore, it must be concluded that, at high acid concentrations the rearrangement of the enamonium tautomer to give the **DH** derivative is much faster than the second peroxo attack.

In summary, in the oxidation of **Y** with PDS the first step of the mechanism is always an electrophilic attack at the C-7 atom of the substrate. The subsequent steps strongly depend on the reaction conditions. Thus, at high acid concentrations the **DH** derivative is the sole reaction product. When the acidity of the media is lowered, a second PDS attack is observed giving the dioxyyohimbine, **Dx**, as the final product. However, as reported by other authors,[12–14] in media of low acid concentrations and in the absence of an electrophilic agent, a rearrangement step, probably through the imine tautomer, is expected to produce a simple oxoindole derivative, **Ox**.

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