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A MECHANISM FOR NITRIC ACID INDUCED OXIDATION PRODUCTS OF YOHIMBANE ALKALOIDS

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The oxidation of two yohimbane alkaloids(reserpine and rescinnamine) by nitric acid has been studied [1,2] and mechanisms are now proposed for the oxidation products. The oxidation pattern of these alkaloids appear to be the same in various organic solvents and the rate may be influenced depending upon the solvent characteristics, i.e. dielectric constant, dissociation constant and viscosity of the medium in the range studied.

Key words. Oxidation, Yohimbane alkaloids, Nitric acid.

Introduction

As a part of our extensive kinetic studies regarding alkaloids, oxidation of reserpine (Ia) and rescinnamine (Ib) by nitric acid in various organic solvents at 25°C were reported earlier [1,2]. The oxidation of these alkaloids follow the first order kinetics and rate of oxidation of these alkaloids appear to depend upon the various solvent characteristics. In this communication, a mechanism for the formation of the oxidation products i.e. 3-dehydroreserpine and 3-dehydro rescinnamine is suggested.

Reserpine is an unstable compound [3] and may decompose in several possible ways into oxidative as well as hydrolytic products [4-6]. It has also been reported [7-8] that it can easily be isomerized and decomposed by air, light and heat. In chloroform solution upon standing, most of the solution acquires pronounced fluorescence [9].

Haycock *et al.* [10] reported nitrous acid oxidation of reserpine in an aqueous solution and expressed the reaction as follows:

Protonated reserpine + HONO $\overset{k_1}{\rightleftharpoons}$ [Protonated reserpine: HONO]

$$\xrightarrow[K_2]{H^+}$$ 3,4-dehydroreserpine (Fluorescent product)

They suggested that only the protonated species are involved in the oxidation reaction.

Hakkesteegt *et al.* [11] presented a reaction scheme for the oxidative degradation of reserpine into 3,4-dehydroreserpine (apple green fluorescence) and 3,4,5,6-tetradehydroreserpine (lumi reserpine) a bright blue fluorescent compound. These oxidation products were also obtained by the oxidation of reserpine in acetic acid with lead tetra acetate as well as by photo oxidation of reserpine in chloroform solution [12]. However, the mechanism for the formation of these oxidation products were not reported. Wenkert *et al.* [13] reported oxidation of a few Rauwolfia alkaloids to 3-dehydro products using mercuric acetate while the mechanism for this reaction was proposed by Leonard *et al.* [14]. Weisenborn and Diassi [15] discovered that normal and allo compounds undergo this oxidation while psendo and epiallo compounds do not undergo such oxidation. These workers described this phenomenon to the ability of an axial C-3 H bond in order to accommodate a coplaner transition state in this reaction mechanism originally proposed by Leonard *et al.* [14].

Materials and Methods

Reserpine and rescinnamine were obtained from CIBA Chemical Company, they were recystallized from CH_3OH before use. The oxidation mixture was prepared by mixing nitric acid (d = 1.4 gm/ml) and glacial acetic acid in 1:1 ratio (v.v). The organic solvents were of analytical grade or of the purest form available from BDH/Merck.

Oxidation of reserpine and rescinnamine. Equimolar solutions of reserpine and rescinnamine (1.2×10^{-4}M) were prepared separately in 50 ml of various organic solvents (formic acid, acetic acid, propionic acid, butyric acid and chloroform) and the oxidation mixture (containing 0.22 to 4.46×10^{-3} M nitric acid) was added to the solution (zero minute). The solution was immediately mixed and placed in 1 cm quartz cell for the absorption measurement at 25 ± 1°C. The absorption spectra were recorded at 1-5 min. intervals using a Schimadzu U-240 recording spectrophotometer and absorbance of each solution was determined around 390 nm.

Determination and identification of 3,4-dehydro products. The oxidised solutions of both alkaloids were subjected to thin layer chromatography using silica gel GF 254 plates, using the solvent systems T_1 (Chloroform-methanol, 40:10 v/v) and T_2 (butanol-butanone-water, 65:25:25 v/v) [12] and the spots were visualized by exposure to UV light (365 nm). The observed R_f values of 3-dehydro reserpine in

$T_1 = 0.64$ and $T_2 = 0.50$ and 3-dehydro rescinnamine, $T_1 = 0.54$ and $T_2 = 0.50$ were recorded whereas R_f values of pure reserpine in these systems are 0.85 and 0.95 and rescinnamine 0.80 and 0.79 respectively.

3,4-Dehydro reserpine was determined spectrophotometrically by its characteristic yellow green fluorescence using a value of 24300 M^{-1} cm^{-1} as the molar absorbtivity at 390 nm [17]. The brown green fluorescence of 3,4-dehydro rescinnamine was determined by the stated method using 22850M^{-1} Cm^{-1}, molar absorbtivity at 389 nm [18]. The validity of Beer's law relation in the concentration range studied was confirmed prior to the analysis in this experiment.

Results and Discussion

In the present kinetic studies of reserpine and rescinnamine (possessing epiallo configuration) the oxidising species is nitronium ion which converts reserpine and ressinnamine into respective 3,4-dehydro products (IIIa) and (IIIb) in accordance with the following mechanism:

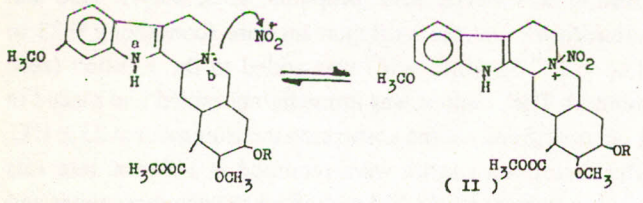
The reaction of nitric acid in acetic acid (oxidising mixture) leads to the formation of nitronium ion by the following reaction [16].

$$H_3CCOOH_3 + HNO_3 \rightleftharpoons CH_3COO^- NO_2^+ + H_2O$$

$$H_3CCOO - NO_2^+ \rightleftharpoons NO_2^+ + CH_3COO^-$$

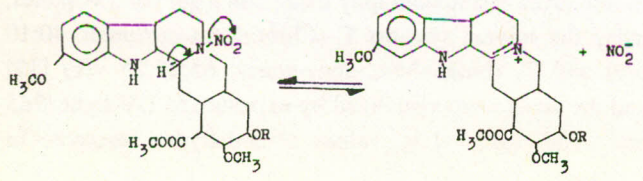
$$\text{or } 2HNO_3 \rightleftharpoons NO_2^+ + NO_3^- + H_2O$$

The nitronium ion acts as an oxidising species and tends to attack more electronegative nitrogen of reserpine and rescinnamine. Under the reaction conditions "N_b of reserpine and rescinnamine seem to be the point of highest electron density and therefore, NO^+_2 preferentially attacks at this point leading to the formation of an intermediate (II).



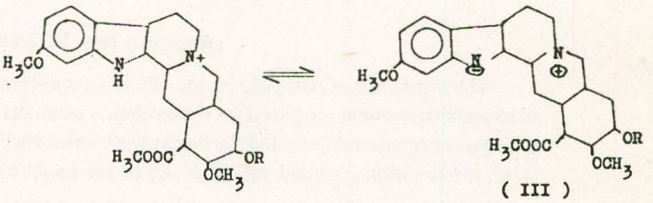
(II)

Concerted electeron shfit appears to take place and the nitrite ion is liberated along with a proton which results in the formation of 3-dehydro products (IIIa) and (IIIb).



R = 3,4,5-trimethoxy benzoyl for reserpine.
R = 3,4,5-trimethoxy cinnamoyl for rescinnamine.

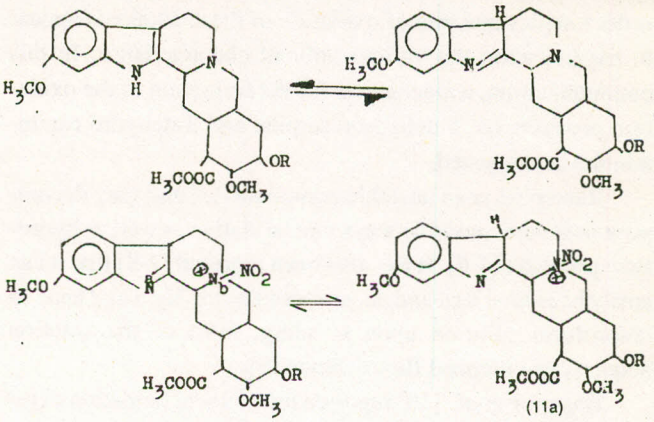
It should be noted that once the positive charge is created on one of the nitrogen atom of reserpine, a negative charge will be created on other nitrogen atom (as zwitter ion formation).



(III)

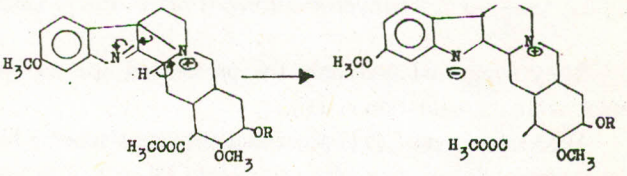
(IIIa) = 3-dehydro reserpine; (IIIb) 3-dehydro rescinnamine

An alternate mechanism may also be proposed. As reserpine and rescinnamine can easily be isomerized under different conditions. The nitronium ion attacks at N_b in this case also forming an intermediate (IIa).



(11a)

Concerted electron shift may take place from C_7 to position 4 to form a bicyclic intermediate IIb with subsequent release of a proton from C-3 to form the desired products (IIIa) and (IIIb) in accordance with the following scheme:



(IIb) (IIIa & IIIb)

It cannot be decided at this stage which one of the above mentioned mechanism are actually operating unless the intermediate are trapped and studied. However, this seems to be the first electronic mechanism for the formation of these oxidation products in presence of nitronium ions. Thus a new field of study for other indole alkaloids containing yohimbane skeleton is now opened. A typical set of absorption spectra

determined during the oxidation of reserpine in $CHCl_3$ is shown in Fig.1.

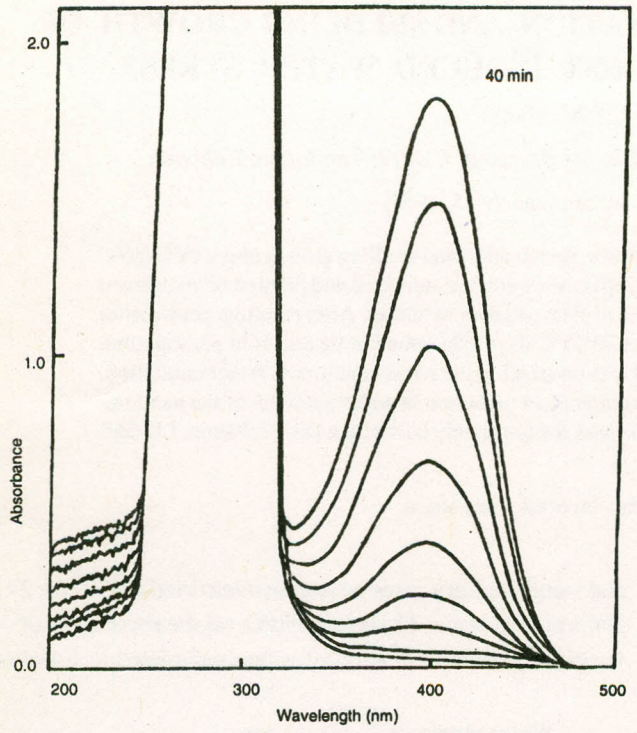


Fig. 1. Absorption spectra of 3, 4-dehydroreserpine measured during the oxidation of reserpine in chloroform, showing a gradual increase in absorbance at 390 nm. The absorption curve was measured at 5 minute interval.

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