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PROTONATION EQUILIBRIA OF RAUWOLFIA ALKALOIDS IN SULFURIC ACID SOLUTIONS

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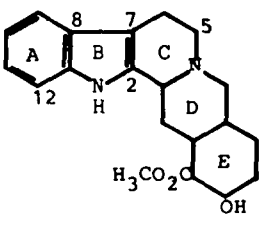
Abstract- The pK_a values for the indole ring protonation equilibria of the Rauwolfia alkaloids, yohimbine, ajmalicine, reserpine and reserpiline have been measured in strongly sulfuric acid solutions. The alkaloids obey the H_I acidity function stablished by Hinman and Lang for indole ring protonation, but they are considerably weaker bases than alkylindoles. Evidences indicating that these compounds behave as carbon bases are reported.

Although the protonation of the indole ring has received considerable attention from theoretical[1-3] and experimental points of view,[4-6] the scope of these investigations has been restricted to simple indole derivatives, while little attention has been paid to other more complex indcles in spite of the biological and pharmaceutical significance of many of them.[7]

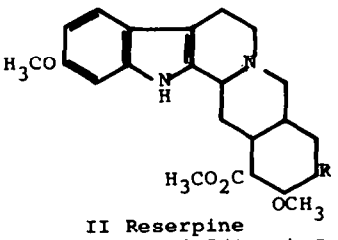
Among the pharmacological active compounds with an indcle nucleous in their structures, Rauwolfia alkaloids are possibly the natural occurring indoles more extensively studied because of their sympatholytic, antihypertensive and sedative properties.[8] Recent interest in these compounds has been stimulated by reports of their ability to destroy selectively and completely the proliferative capacity of many different cancer cells in vitro.[9,10]

It has been reported that a considerable number of typical reactions,[11] within this family of compounds, such as epimerizations,[12] electrophilic substitution and oxidative rearrangements are initiated[13-15] or greatly influenced by protonation of the indole nucleous. However, data are not available about this protonation equilibria. In view of the paucity of data, the principal aim of this paper is to present a study of the protonation equilibria of several of these alkaloids (SchemeI)

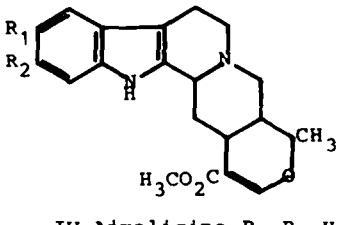
SCHEME I



I Yohimbine



II Reserpine
R=3,4,5$(OCH_3)_3C_6H_2COO$-



IV Ajmalicine $R_1=R_2=H$
V Reserpiline $R_1=R_2=OCH_3$

in strongly sulfuric acid solutions. Also, 2,3-dimethylindole has been included in this study as a model compound.

EXPERIMENTAL

Rauwolfia alkaloids were kindly donated by Boehringer and Sohn and 2,3-dimethylindole was purchased from EGA-Chemie. All materials were of the best available commercial quality and were used without further purification. Stock solutions, 10^{-3}M were prepared in methanol. Sulfuric acid solutions were prepared by dilution with distilled water of Merck sulfuric acid R.A. and concentrations were checked by titration of suitably diluted solutions. Aliquots of each indole solutions (100-200γl) were pippeted into 5cm^3 of sulfuric acid solutions which were kept in a constant temperature bath at 25.0±0.1°C.

After thouroughly mixing, UV-spectra and absorbance measurements of the solutions were made on a Lambda-5 spectrophotometer whose cell compartment was also thermostatted at 25.0±0.1°C. Usually, the solutions in sulfuric acid were sufficiently stable for spectrophotometric measurements, the UV-spectra changed only slightly over 5-10min and were not significantly dependent on careful exclusion of air. When the solutions were unstable, the absorbances were recorded as a function of time and values at zero time determined by lineal extrapolation. It is to be noted the fluorescent properties exhibited for the more concentrated sulfuric acid solutions of some alkaloids.

RESULTS AND DISCUSSION

Two series of spectrophotometric measurements were performed with each alkaloid. In the first series, the detailed spectra were recorded at a few sulfuric acid concentrations in order to determine the more suitable wavelengths for the determination of the protonation ratio I(I=$|BH^+|/|B|$).

It was observed that unprotonated species showed two main absorption bands, with maxima near to 220nm and 280-290nm respectively. Both bands are typicals of indole compounds. Protonation was accompanied by a marked decrease in the intensity of the absorption maximum near to 220nm and by a bathochromical shift of the latter band. The first two columns in Table1 show the position of the absorption maxima, λ_{max}, and into parenthesis, the approximative extinction coefficients for both, the protonated and unprotonated species.

TABLE1: Absorption characteristics and pK_{BH^+} values of Rauwolfia alkaloids

	UV-spectra data		
	B λ_{max} (log ε)	BH^+ λ_{max} (log ε)	pK_a
2,3-dimethylindole	225(4.5), 279(4.0)	231(4.0), 281(4.0)	-1.49±0.05
Yohimbine	219(4.5), 272(3.9)	235(4.0), 308(3.8)	-8.30±0.04
Ajmalicine	220(4.5), 248(3.9) 292(3.4)	269(4.1), 310(3.8)	-8.31±0.04
Reserpine	216(>4.8),268(4.2) 295(4.0)	269(4.2), 305(4.3)	-9.26±0.05
Reserpiline	229(4.6), 300(4.0)	267(4.2), 315(4.2)	-7.26±0.03

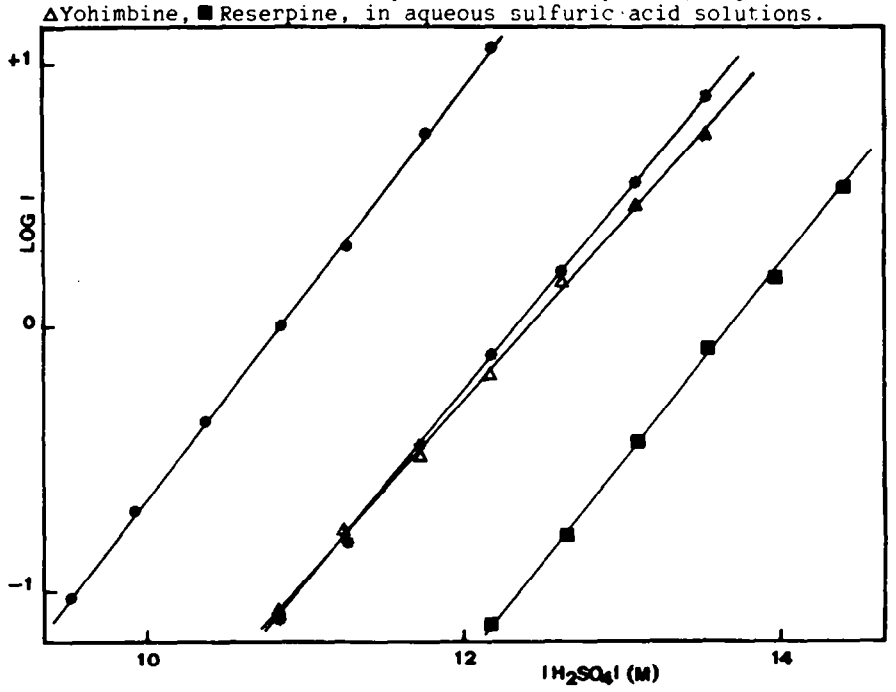
A second series of spectrophotometric measurements were then performed at a large number of sulfuric acid concentrations, but only at three wavelengths around the maxima near to 300nm. Titration curves are shown in Fig.1. The average value of $d\log I/d|H_2SO_4|$ is 0.72±0.03 which closely approaches the average value previously reported for alkylindoles[5] (0.70±0.03).

On the other hand, semilogarithmic plots of the protonation ratio of each alkaloid against the H_o (Hammet) and H_I (Hinman y Lang)[5] acidity functions were both reasonably linears with average slopes of 1.21±0.04 and 1.02±0.04 respectively. Therefore, the H_I function stablished by Hinman and Lang for the indole ring protonation seems to be the best function correlating the protonation of the Rauwolfia alkaloids and hence, the pK_a values have been calculated using the following standard equation,

$$pK_a = H_I + \log I \qquad (1)$$

Because of the H_I function did not extend sufficiently to accomodate our data, the

FIGURE1: Protonation ratio plots of: ● Reserpiline, ● Ajmalicine, △Yohimbine, ■ Reserpine, in aqueous sulfuric acid solutions.



necessary H_I values were obtained by linear extrapolation. The proportionality between H_I values and sulfuric acid concentrations over the most part of the acidity range and, on the other hand, the agreement between the average slopes of the titrations curves of the alkaloids and alkyilindoles support this procedure. The results of pK_a determinations are reported in Table1. It can be noted that the pK_a value of 2,3-dimethylindole is in excellent agreement with the previously reported by Hinman and Lang.[5]

Results in Table1 show that Rauwolfia alkaloids are much weaker bases than 2,3-dimethylindole. Although alkyl substitution on C-3 of indole ring has a base weakening effect, the differences on pK_a values are particularly noteworthy. Similar differences have been observed with triptamines (triptamine has a pK_a of 6.31) and they have been attributed to the strong electrostatic destabilizing effect of the positive charge on the exocyclic nitrogen atom in the further indole ring protonation.[16] The nonindolic nitrogen atom of Rauwolfia alkaloids is far more basic than the indole ring and therefore, a similar effect could be considered.

Although the main subject of this paper is concerned with the determination of the pK_a values for protonation on the indole ring, the results obtained provided some evidences regarding the site of protonation. Firstly, the fact that Rauwolfia alkaloids obey the H_I function suggests that indole ring protonation takes place on carbon in preference to nitrogen. Thus, pyrroles[17] and indoles[6] which behave preferentially as carbon bases, and undergo protonation on the α and β carbon atoms respectively, obey the H_I function, while carbazoles[18] which are protonated on nitrogen atom do not obey this function and deviate only slightly from H_o.

On the other hand, it should be noted that the C-7 position (β-position in relation to the indole ring) has long been recognized as the preferred site for electrophilic substitution reactions of Rauwolfia alkaloids.[11-15] Therefore, if protonation follows this general pattern, the protonated species would have an indoleninium structura and their UV-spectra might be expected to resemble those of the derivatives of Rauwolfia alkaloids with indolenine structure.

The UV-spectra of these latter compounds[14,15,19] show bands with maxima around to 266nm and 295nm. An inspection of the spectra of the protonated alkaloids shows

that, as expected, the band at 266nm appears upon protonation of ajmalicine and reserpiline, but it is absent in yohimbine and it is not significative in reserpine because of this maximum remains at about the same place and intensity in the unppotonated alkaloid.

Moreover, the bands near to 305-315nm in protonated species are 10-20nm bathochromically shifted with respect to those of the indolenines. These absorptions at longer wavelengths seem to involve a longer conjugated systems extending over the other rings in the molecule. It is to be noted that similar bathochromic shifts have been observed with 2-arylindoles[20] which upon protonation can incorporate a benzylideneaniline chromophore.

By analogy, an alkylidene system could be assumed here as responsible of the observed bathochromic displacements. In fact, mechanisms involving 2-alkylidene indole type intermediates have been proposed for most of the typical reactions of the Rauwolfia alkaloids. On the other hand, the longer wavelength absorption of protonated reserpiline can be explained by considering that the 10-methoxy substituent is conjugated with the alkylidene chromophore. The pK_a values can be explained in a similar way.

Therefore, although it is tempting to suggest that protonation takes place on the 7-position, it does not imply that all the alkaloids are converted exclusively to indoleninium cations, but that other structures might be taken into account. A more detailed examination of the protonation site of Rauwolfia alkaloids will be the subject of a further investigation.

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