Determination of Individual Proton Affinities of Reserpine from Its UV−Vis and Charge-Transfer Spectra

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Proton affinities of the two N atoms of reserpine (methyl-11,17α-dimethoxy-18β-[(3,4,5-trimethoxybenzoyl)oxy]-3β,20α-yohimban-16β-carboxylate) have been determined in two ways from the pH-dependent variation of the UV−vis absorption spectra (i) of reserpine itself and (ii) of the charge-transfer (CT) spectra of its complexes with *o*-chloranil, *p*-chloranil, and DDQ in aqueous medium (containing 0.1% ethanol v/v). For the second method, the CT absorption bands of the complexes were determined, their formation constants were estimated by a modified Benesi−Hildebrand equation, and variation of CT absorption spectra with a change in pH was noted. A necessary working formula for the second method was derived and utilized with the experimental data. The pK_a values obtained by the two methods are well in agreement with each other within the limits of experimental error. To our knowledge, so far, this is the first report on determination of pK_a from charge-transfer complex formation in aqueous solution using simple absorption spectroscopy in the UV−vis region. The results obtained were further checked by noting the variation of fluorescence intensity of reserpine upon addition of *o*-chloranil, acid, and base, and almost complete agreement with the absorption spectrometric result was observed.

1. Introduction

Study of electron (charge)-transfer processes in biomolecular systems is a topic of current interest.[1–6] Thus, by single-molecule spectroscopy, it has been reported[1] that DNA charge transfer highly depends on the electronic interaction between base pairs and reflects the difference in base composition and sequence. Fast photoinduced electron transfer through the DNA helix has been studied by Murphy et al.[2] using an electron-donor Ru(II) complex and an electron-acceptor Ru(III) complex as intercalators into DNA with a high binding constant. Barry et al.[3] have recently studied a proton-coupled electron transfer in a biomimetic peptide as a model of the enzyme regulatory mechanism. Charge transfer from tyrosine to the tryptophan radical in bovine milk casein has been studied[4] by pulse radiolysis. In bacterial adhesion to conducting surfaces, it has been shown[5] by using *Staphylococcus epidermidis* 3399 and titaniumoxynitride substrata that the electron (charge)-transfer process plays an important role. It is thus evident that studies of charge-transfer processes are of great importance in both biomolecular and material science. Recently,[6] novel fluorescence pH sensors are being developed for determination of the pK_a of biomolecular acids. In the present article, spectra of charge-transfer complexes are shown to be useful for the determination of the pK_a of methyl-11,17α-dimethoxy-18β-[(3,4,5-trimethoxybenzoyl)oxy]-3β,20α-yohimban-16β-carboxylate (structure I, Figure 1), which is commonly called reserpine. It is used as an antihypertensive drug. By preventing the active uptake of catecholamine into the synaptic vesicles, reserpine results in depletion of catecholamine and thus inhibits the vesicular catecholamine pump. This is believed to be responsible for the antihypertensive action of reserpine.[7] It is an alkaloid occurring in the roots of *Rauwlfia serpintina.* The reserpine molecule contains two types of N atoms; one is of the indole type (sp^2-hybridized), and the other is a tertiary N (sp^3-hybridized). Their proton affinities, expressed in terms of pK_a values of the conjugate acids, are important clinical parameters for a clear understanding of the mechanism of action of reserpine as a drug. Individual values of pK_a are

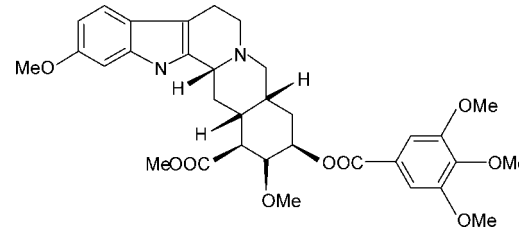

Figure 1. Structure of reserpine.

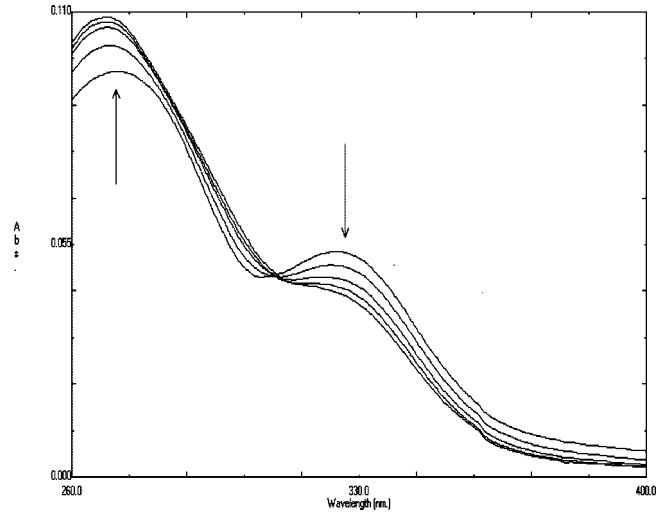

Figure 2. Variation of absorption spectrum of aqueous reserpine solution (≈10^{-6} M) with changes in pH in the acidic range. The arrows indicate the direction of the change of intensity at the two peaks (292 and 326 nm) with a gradual decrease in pH.

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TABLE 1: Variation of the Absorbance of Reserpine Solution at 326 nm with Changes in pH (Temp = 298 K)

pH	absorbance	log{[acid]/[conj.base]}	pK_a
4.26	0.0447	−0.0662	$(pK_a)_1 = 4.22 \pm 0.01$
4.31	0.045	−0.0948	
4.37	0.0457	−0.1624	
4.44	0.0466	−0.2523	
4.5	0.048	−0.4046	
4.56	0.0481	−0.4163	
4.71	0.0499	−0.6605	
5.37	0.0526	−1.4723	
5.67	0.0529	−1.7806	
5.97	0.0531	−2.2625	
9.64	0.0585	0.38782	$(pK_a)_2 = 9.97 \pm 0.01$
9.82	0.0605	0.17713	
9.94	0.0625	−0.0145	
10.11	0.0638	−0.1419	
10.24	0.0654	−0.311	
10.54	0.0681	−0.6498	
10.64	0.0687	−0.7647	

not well established; only an overall pK_a is reported in the literature.[8] In acidic pH ranges, reserpine exists in two forms, $ResH^+$ (by protonation at the tertiary N) and $ResH_2^{2+}$ (by protonation of both the tertiary and indolic N). In the present work, the individual pK_a values, namely, $(pK_a)_1$ for the deprotonation of $ResH_2^{2+}$ from the indolic N site and $(pK_a)_2$ for the deprotonation of $ResH^+$ from the tertiary N, have been determined from spectral variation of an aqueous reserpine solution with pH. Moreover, in the present work, reserpine is shown to form charge-transfer (CT) complexes[9,10] with the known electron acceptors,[11] namely, *o*-chloranil, *p*-chloranil, and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ). In these complexes, the indolic N acts as the electron-donor center. From the variation of the CT absorption band of the reserpine−*o*-chloranil complex with changes in pH, the $(pK_a)_1$ of reserpine corresponding to the indolic N protonation has been determined. As reserpine itself is fluorescent, quenching experiments were also performed to check the result obtained by absorption spectrometry. Although CT complexes have found many important applications in semiconductivity,[12] photocatalysis,[13] and drugs and pharmaceuticals,[14–17] to our knowledge, this is the first attempt to determine the pK_a from the CT absorption band of a molecular complex.

2. Materials and Methods

Reserpine was collected from Fluka, and *o*-chloranil, *p*-chloranil, and DDQ were collected from Aldrich and were used without further purification. Ethanol was purified by a standard method.[18,19] The main solvent, water, was double distilled just before use. Aqueous solutions ($\approx 10^{-6}$ M) of reserpine could be prepared by direct weighing and dissolving in water. However, the quinones, which are highly insoluble in water, were dissolved in freshly purified ethanol, and microliter quantities of the solutions were added to an aqueous reserpine solution by a Hamilton microsyringe to get the desired quinone−reserpine mixture of appropriate concentration. The resulting mixtures were nearly completely aqueous (≈0.1% ethanol v/v). Spectral measurements were carried out using a UV 1601 PC model Shimadzu spectrophotometer fitted with a Peltier-controlled thermo bath. The fluorescence study was carried out using a Hitachi F-4500 fluorescence spectrophotometer.

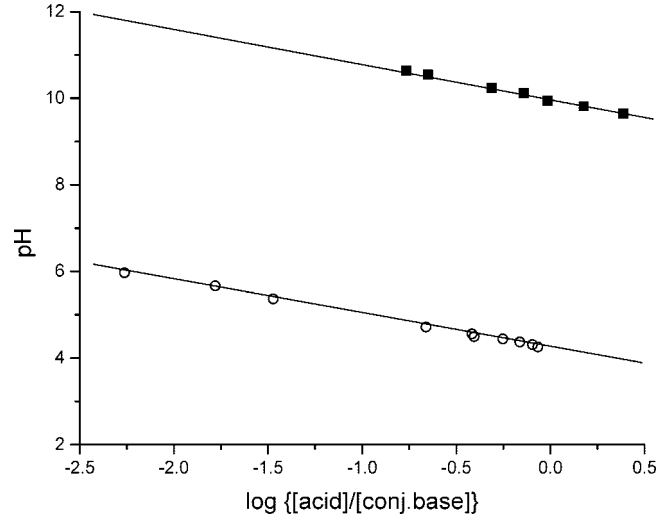

Figure 3. Plot of pH against $\log\{[A_j - A_{max}]/[A_{min} - A_j]\}$ in the acidic range (hollow circle) and against $\log\{[A_{min} - A_j]/[A_j - A_{max}]\}$ in the alkaline range (solid square).

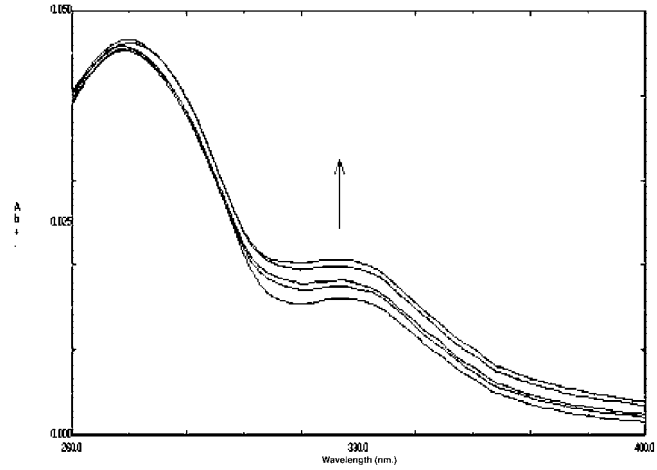

Figure 4. Variation of the absorption spectrum of an aqueous reserpine solution ($\approx 10^{-6}$ M) with changes in pH in the alkaline range. The arrows indicate the direction of the change of intensity at the 326 nm peak with a gradual increase in pH.

3. Results And Discussion

3.1. Absorption Spectrophotometric Determination of pK_a Corresponding to the Indolic Nitrogen. Figure 2 shows the variation of the absorption spectrum of aqueous reserpine solution ($\approx 10^{-6}$ M) with changes in pH. In aqueous solution, reserpine shows two absorption peaks at 326 and 292 nm. With gradual addition of HCl, the intensity at the 326 nm peak systematically decreases while that at the 292 nm peak increases, showing an isosbestic point at 308 nm. Since the tertiary N atom is nearly as basic as ammonia,[20] reserpine in pure water solution exits as $ResH^+$, in which the tertiary N atom is protonated. When HCl is added, the indolic N becomes protonated, and the lone pair of electrons of this N become less available for π-conjugation in the aromatic part of the molecule. This causes a decrease in intensity of the longer wavelength peak (326 nm) and an increase in intensity of the shorter wavelength peak (292 nm). Such varying absorption intensities at these two wavelengths reach respective limiting values at high acid concentration, indicating complete protonation of the indolic N. These observations together with the appearance of the isosbestic point suggest that in acidic aqueous solution, the following equilibrium exists

$$ResH_2^{2+} + H_2O \rightleftharpoons ResH^+ + H_3O^+ \quad (1)$$

where $ResH_2^{2+}$ means reserpine with both of its N atoms protonated. We can, therefore, assume that

$$[ResH^+]_{total} \propto A_{max} \qquad [ResH_2^{2+}]_{total} \propto A_{min} \quad (2)$$

where A_{max} and A_{min} are the maximum and minimum intensities at 326 nm.

The $(pK_a)_1$ of reserpine corresponding to the equilibrium in eq 1 can now be calculated from the following equation

$$pH = (pK_a)_1 - \log\frac{[ResH_2^{2+}]}{[ResH^+]}$$

$$= (pK_a)_1 - \log\frac{[A_j - A_{max}]}{[A_{min} - A_j]} \quad (3)$$

where A_j is the absorbance of the jth solution of the absorption intensity between the two extremes.

Results are shown in the first part of Table 1. The plot of pH against $\log[ResH_2^{2+}]/[ResH^+]$ is shown in Figure 3. The plot is excellently linear according to eq 3, with $r^2 = 0.99$. From

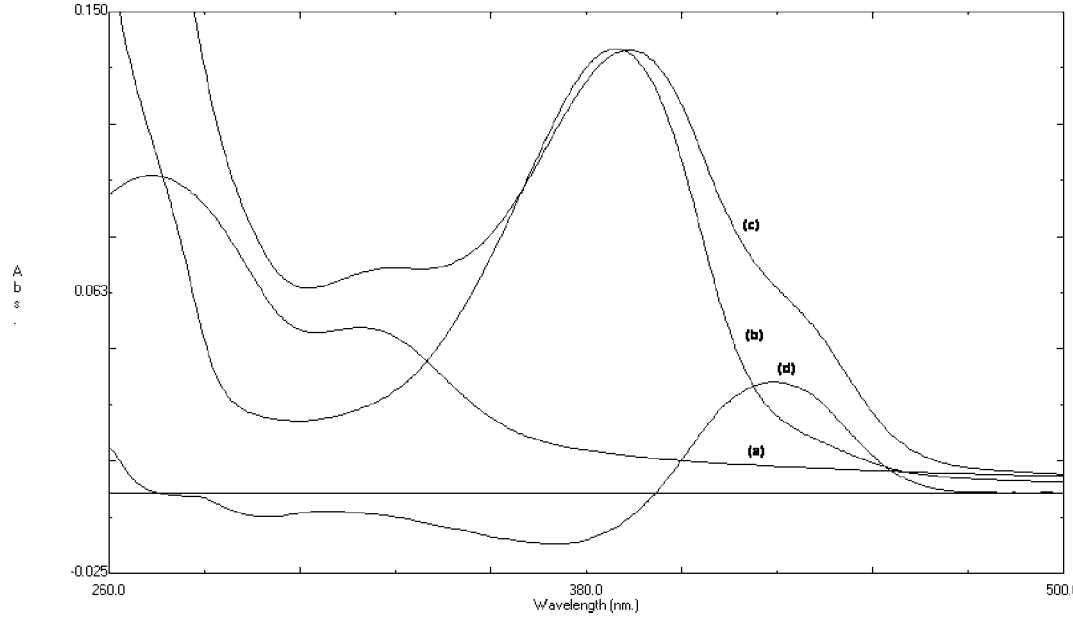

Figure 5. Absorption spectra of (a) free reserpine (in pure water medium, [Res] = 6.21×10^{-6} mol dm^{-3}), (b) DDQ (conc. = 20×10^{-6} mol dm^{-3}) in very nearly aqueous medium (≈0.1% ethanol−water v/v), and (c) a mixture of DDQ and reserpine in the same medium with concentrations as those in (a) and (b). The CT spectrum (d) of the reserpine−DDQ complex was obtained by the difference method.

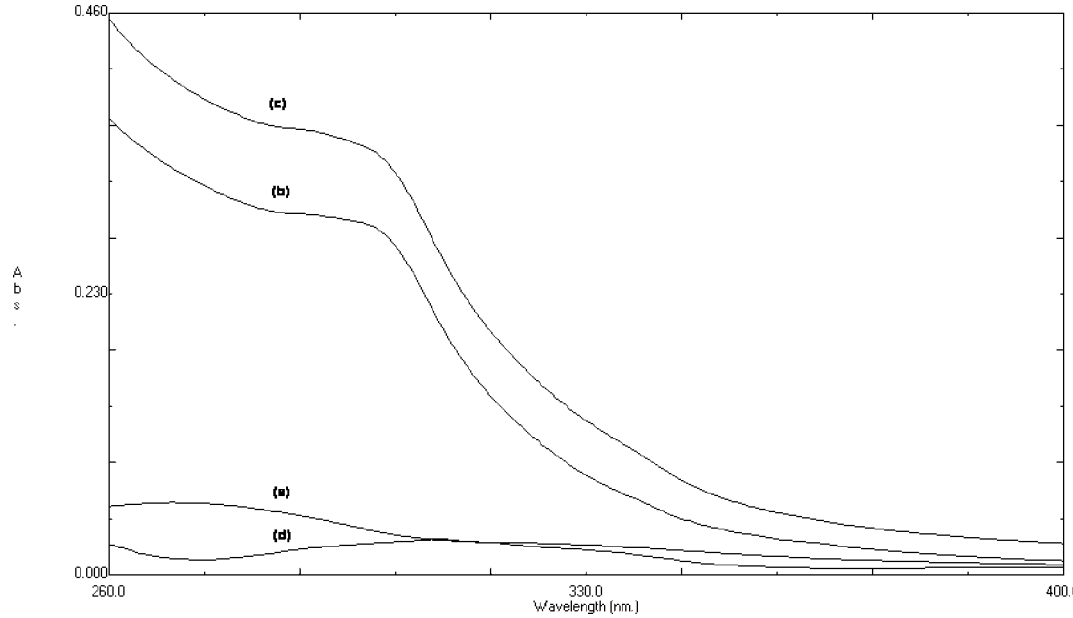

Figure 6. Absorption spectra of (a) free reserpine (in pure water medium, [Res] = 2.93×10^{-6} mol dm^{-3}), (b) *o*-chloranil (conc. = 9.797×10^{-5} mol dm^{-3}) in very nearly aqueous medium (≈0.1% ethanol−water v/v), and (c) a mixture of *o*-chloranil and reserpine in the same medium with concentrations as those in (a) and (b). The CT spectrum (d) of the reserpine−*o*-chloranil complex was obtained by the difference method.

TABLE 2: Data for Determination of the Formation Constant of the Reserpine−DDQ Charge-Transfer Complex in Aqueous Medium (≈0.1% Ethanol v/v)[a]

10^6 $[A]_0$ (mol dm^{-3})	absorbance of the mixture	10^9 $[A]_0[D]_0/d'$	10^5 $([A]_0+[D]_0)$	formation constant (K)	ε_C
2.0	0.016	1.57215	0.821		
4.0	0.0249	1.63421	1.021		
6.0	0.0289	2.11705	1.221		
8.0	0.0365	2.10508	1.421		
10.0	0.0387	2.56612	1.621	$(4.07 \pm 0.02) \times 10^5$ dm^3 mol^{-1}	7441
12.0	0.046	2.49231	1.821		
16.0	0.0496	3.27921	2.221		
20.0	0.0529	4.03247	2.621		

[a] Concentration of reserpine, $[D]_0 = 6.21 \times 10^{-6}$ mol dm^{-3}. Absorbances were measured at 438 nm. Temp. = 298 K.

TABLE 3: Data for Determination of the Formation Constant of the Reserpine−*o*-Chloranil Charge-Transfer Complex in Aqueous Medium (≈0.1% Ethanol v/v)[a]

10^6 $[A]_0$ (mol dm^{-3})	absorbance of the mixture	10^9 $[A]_0[D]_0/d'$	10^5 $([A]_0+[D]_0)$	formation constant (K)	ε_C
1.0	0.0618	3.75641	1.293		
2.0	0.0955	4.04138	2.293		
3.0	0.1155	4.53093	3.293		
4.0	0.1318	5.30317	4.293	$(1.68 \pm 0.01) \times 10^4$ dm^3 mol^{-1}	30775
5.0	0.1537	5.70039	5.293		
6.0	0.1769	5.95932	6.293		
7.0	0.1918	6.79139	7.293		

[a] Concentration of reserpine, $[D]_0 = 2.93 \times 10^{-6}$ mol dm^{-3}. Absorbances were measured at 309 nm. Temp. = 298 K.

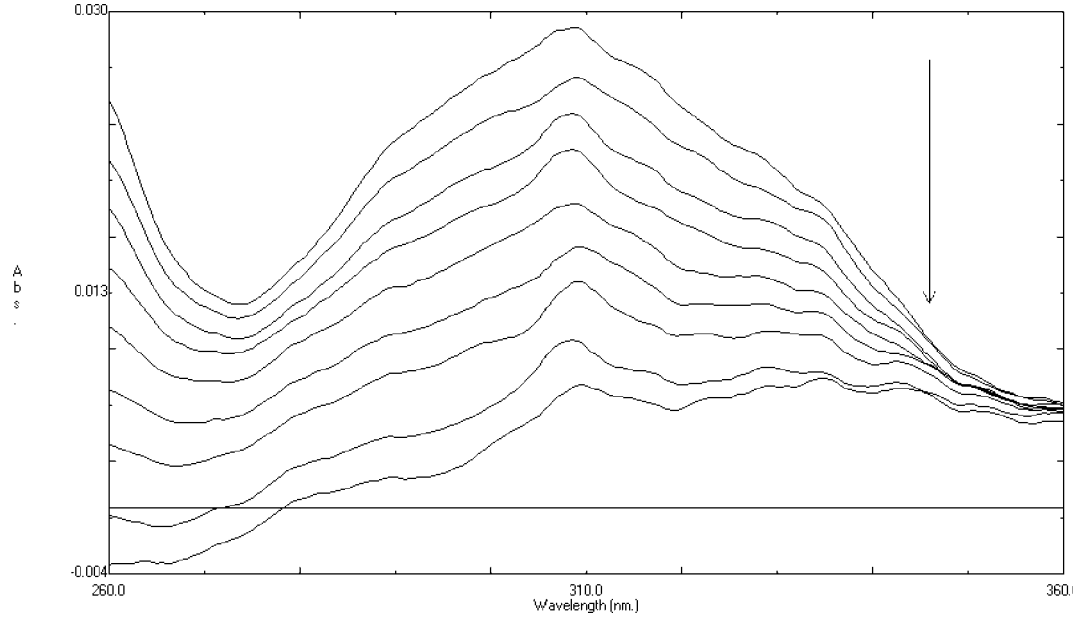

Figure 7. Spectral variation of the reserpine−*o*-chloranil CT complex with changes in pH. The arrow indicates the direction of decrease in pH.

the intercept, we obtained $(pK_a)_1 = 4.22 \pm 0.01$, which is characteristic of indolic nitrogen.[20]

3.2. Absorption Spectrophotometric Determination of pK_a Corresponding to the Tertiary Nitrogen. Figure 4 shows the variation of the absorption spectrum of an aqueous reserpine solution with gradual addition of NaOH solution; the intensity at the 326 nm peak increases systematically with addition of NaOH. However, above pH ≈ 11, the free reserpine precipitates out, making spectral measurement impossible at or above such high pH. In this case

$$[ResH^+]_{total} \propto A_{min} \qquad [Res]_{total} \propto A_{max} \tag{4}$$

The $(pK_a)_2$ here corresponds to the equilibrium

$$ResH^+ + H_2O \rightleftharpoons Res + H_3O^+ \tag{5}$$

where Res means free, that is, unprotonated reserpine.

Here

$$pH = (pK_a)_2 - \log\frac{[ResH^+]}{[Res]}$$

$$= (pK_a)_2 - \log\frac{[A_{min} - A_j]}{[A_j - A_{max}]} \tag{6}$$

Experimental data are shown in the second part of Table 1. The plot of pH against log $[ResH^+]/[Res]$ is shown in Figure 3, where a similar plot for the acidic range is also shown. The plot for the alkaline range is again linear, as expected from eq 6, with $r^2 = 0.99$. The intercept gives $(pK_a)_2 = 9.97 \pm 0.01$, which is characteristic of tertiary nitrogen.[20] It is to be noted that the two straight lines are parallel with unit slope as required by eqs 3 and 6.

3.3. Observation of CT Absorption Bands of Complexes of Reserpine with DDQ, *p*-Chloranil, and *o*-Chloranil and the Determination of the Formation Constants of the Complexes. Figure 5 shows the absorption spectra of free reserpine (in pure water medium), DDQ in very nearly aqueous medium (≈ 0.1% ethanol−water v/v), and of a mixture of DDQ and reserpine in the same medium. The CT absorption spectrum was obtained by the subtraction method; from the spectrum of the reserpine−DDQ mixture, the sum of the spectra of the individual components with concentrations equal to those in the mixture was subtracted. The CT peak appeared at 422 nm. At 387 nm, the subtraction spectrum shows negative absorption because some DDQ (whose $\lambda_{max} = 387$ nm) has taken part in complexation. By a similar method, the CT peaks of complexes of reserpine with *p*-and *o*-chloranil were found to be, respectively, at 281 and 309 nm. The CT spectrum of the reserpine−*o*-chloranil complex obtained by the difference method is shown in Figure 6. To verify that these are really CT peaks, the vertical electron affinities (E_A^v) of the quinone-type acceptors were plotted against the transition energies ($h\nu_{CT}$) corresponding to the wavelengths at these peaks. As expected from Mulliken's theory,[9,10] a parabolic plot (Figure S1 of the Supporting Information) was obtained with the regression equation

$$E_A^{\ v} = 2.91768 + 1.87379(h\nu_{CT}) - 0.57108(h\nu_{CT})^2 \qquad r^2 = 0.99 \quad (7)$$

A similar relation with justification from Mulliken's theory was observed in a previous work[21] for a series of CT complexes of a crown ether (donor) and quinone-type acceptors and fullerenes. By varying the concentration of the acceptor in a fixed reserpine solution, the formation constants were determined by using a modified form of the Benesi−Hildebrand[22] equation

$$\frac{[A]_0[D]_0}{d'} = \frac{([D]_0 + [A]_0)}{\varepsilon'} + \frac{1}{K\varepsilon'} \qquad (8)$$

where $[A]_0$ and $[D]_0$ are the initial concentrations of the acceptor (DDQ or *o*-chloranil) and donor (reserpine), respectively, and K is the formation constant of the complex. The primed quantities are defined as follows

$$d' = (d - d_A^{\ 0} - d_D^{\ 0}) \qquad \varepsilon' = (\varepsilon_C - \varepsilon_A - \varepsilon_D) \qquad (9)$$

where d is the absorbance of the donor−acceptor mixture at the wavelength (λ) of measurement, $d_A^{\ 0}$ and $d_D^{\ 0}$ are the absorbances of the acceptor and donor, respectively, at the same λ with their concentrations equal to those in the mixture, and ε_C, ε_A, and ε_D are, respectively, the molar absorptivities of the complex, acceptor, and donor at wavelength λ. Equation 8 is valid for complexes of 1:1 stoichiometry. Experimental data are shown in Table 2 for DDQ and in Table 3 for *o*-chloranil. These data give excellent linear correlation, as expected from eq 8

For the reserpine−DDQ complex

$$\frac{[A]_0[D]_0}{d'} = (1.34387 \pm 0.10799) \times 10^{-4}([D]_0 + [A]_0) + (3.30012 \pm 0.18300) \times 10^{-10} \quad (10)$$

For the reserpine−*o*-chloranil complex

$$\frac{[A]_0[D]_0}{d'} = (5.03939 \pm 0.29162) \times 10^{-5}([D]_0 + [A]_0) + (2.99130 \pm 0.13811) \times 10^{-9} \quad (11)$$

Figures S2 and S3 of the Supporting Information demonstrate the linear plots according to eq 8, with correlation coefficients of 0.98 and 0.99, respectively. From the slopes and intercepts, the values of K and ε_C were calculated. Results are shown in Tables 2 and 3. It is found that fairly strong complexes (with stability constants ≈ 10^5 dm^3 mol^{-1} for the DDQ acceptor and ≈ 10^4 dm^3 mol^{-1} for the *o*-chloranil acceptor) with a stoichiometry of 1:1 are formed in each case.

TABLE 4: Data for Determination of $(pK_a)_1$ from Variations of the Spectra of the Reserpine−*o*-Chloranil Charge-Transfer Complex with Changes in pH[a]

pH	absorbance	log Z	pK_a
4.15	0.0219	0.61211	
4.08	0.0204	0.66192	
4.02	0.0186	0.7238	
3.97	0.017	0.78134	4.75
3.92	0.0161	0.81503	
3.88	0.0149	0.86175	
3.84	0.0139	0.90252	
3.81	0.0128	0.94971	

[a] Concentration of reserpine, $[Res]_0 = 2.93 \times 10^{-6}$ mol dm^{-3}. Concentration of *o*-chloranil, $[A]_0 = 9.797 \times 10^{-5}$ mol dm^{-3}. Absorbances were measured at $\lambda_{CT} = 309$ nm. Temp = 298 K.

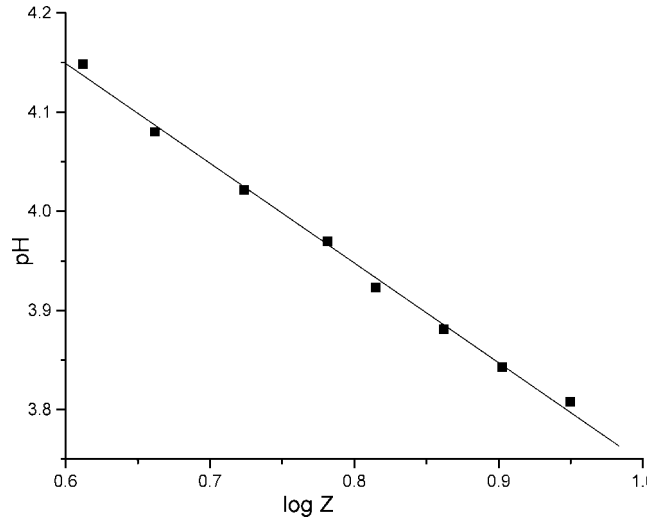

Figure 8. Plot for the determination of the $(pK_a)_1$ of reserpine from variations of the CT absorption intensity of the reserpine−*o*-chloranil complex with changes in pH.

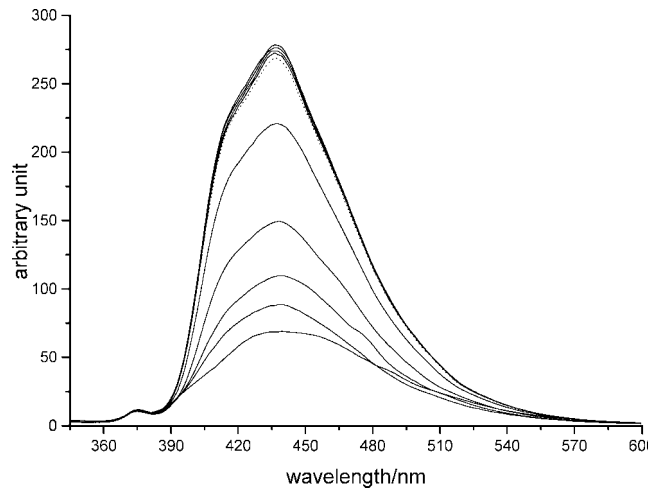

Figure 9. Variations of the emission spectra of reserpine with changes in pH. The dotted curve represents the fluorescence spectrum of reserpine in aqueous solution (≈10^{-6} M). The fluorescence intensity increases from the lowermost to the uppermost value for a change in pH from 1.5 to 10.5.

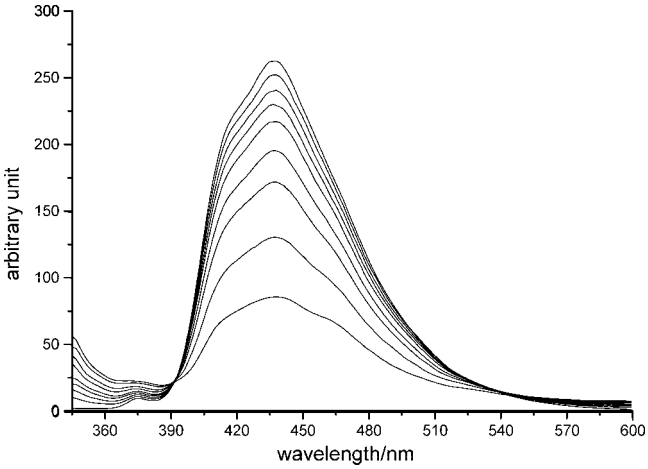

Figure 10. Quenching of reserpine fluorescence by *o*-chloranil.

TABLE 5: Data for Determination of Formation Constant (*K*) of the Reserpine−*o*-Chloranil Charge-Transfer Complex by the Fluorescence Quenching Experiment[a]

$10^6 \times$ [Q] (mol dm^{-3})	fluorescence intensity (au)	K (dm^3 mol^{-1})
2.5	251.8	
5.0	240.9	
7.5	229.8	
10	217.2	$(1.49 \pm 0.02) \times 10^4$
12.5	207.5	
15	195.7	
20	172.1	
25	153.5	

[a] Concentration of reserpine, $[\text{Res}]_0 = 4.87 \times 10^{-6}$ mol dm^{-3}. The quencher (Q) is *o*-chloranil. $\lambda_{ex} = 330$ nm, $\lambda_{em} = 437$ nm. Temp = 298 K.

3.4. Determination of pK_a for the Indolic N of Reserpine from Variation of the CT Absorption Spectra of the Complexes with Change In pH. In an acidic aqueous medium containing reserpine and a quinone-type acceptor (A), the following equilibria exist

$$\text{ResH}_2^{2+} \rightleftharpoons \text{ResH}^+ + \text{H}^+ \tag{12}$$

$$\text{ResH}^+ + \text{A} \rightleftharpoons \text{ResH}^+ \cdot \text{A} \qquad \text{(CT complex)} \tag{13}$$

For eq 12, the dissociation constant of the diprotonated reserpine is given by

$$K_a = \frac{[\text{ResH}^+] \cdot [\text{H}^+]}{[\text{ResH}_2^{2+}]} \tag{14}$$

$$\text{pH} = (\text{p}K_a)_1 - \log \frac{[\text{ResH}_2^{2+}]}{[\text{ResH}^+]} \tag{15}$$

For the formation of the equilibrium in eq 13, the total reserpine concentration is given by

$$[\text{Res}]_0 = [\text{ResH}^+] + [\text{ResH}_2^{2+}] + [\text{ResH}^+ \cdot \text{A}] \tag{16}$$

The formation constant of the CT complex is

$$K = \frac{[\text{ResH}^+ \cdot \text{A}]}{[\text{ResH}^+] \cdot [\text{A}]} \tag{17}$$

Utilizing eqs 14–17, one can show that

$$\text{pH} = (\text{p}K_a)_1 - \log Z \tag{18}$$

where

$$Z = \frac{[\text{Res}]_0 - \left(1 + \dfrac{1}{K[\text{A}]}\right)[\text{ResH}^+ \cdot \text{A}]}{\dfrac{[\text{ResH}^+ \cdot \text{A}]}{K[\text{A}]}} \tag{19}$$

When acid is added to the reserpine−quinone mixture, the absorption intensity of the CT complex decreases, and at the same time, that of the free acceptor increases. This is shown in Figure S4 of the Supporting Information for the reserpine−DDQ complex as a typical case. Such variation of the CT spectrum with pH indicates that the added proton is taken up by the indolic N of reserpine and the acceptor (quinone) is set free from the complex. The isosbestic point at 408 nm in the CT spectrum of the reserpine−DDQ complex (Figure S4 of the Supporting Information) supports this fact. However, the determination of pK_a could not be performed with the DDQ complex because the acid concentration could not be raised to an appreciable extent. Figure S4 was obtained only in the narrow pH range of 6−7. DDQ has a fairly high proton affinity, and below pH ≈ 6, DDQ and indolic N compete with each other for accepting the added proton. In acidic solution, DDQ and its protonated form exhibit an entirely different spectra with an isosbestic point at 292 nm. To avoid such complications, we attempted to determine $(\text{p}K_a)_1$ of reserpine with the *o*-chloranil complex. Spectral variation of the reserpine−*o*-chloranil CT complex with changes in pH is shown in Figure 7. By applying the working formula in eq 18 to the data shown in Table 4, we obtained an excellent linear plot (Figure 8) with $r^2 = 0.998$. The intercept gives $(\text{p}K_a)_1 = 4.75 \pm 0.20$, which is close to the value (4.22 ± 0.01) obtained from spectral variation of aqueous reserpine solution alone with changes in pH.

3.5. Fluorimetric Determination of pK_a Values Corresponding to the Indolic and Tertiary Nitrogen Atoms. Reserpine is fluorescent, and when excited at 330 nm, it emits at 437 nm. By using the same concentration of reserpine as that in the absorption experiment and gradually lowering the pH by addition of HCl, no shift in the emission peak was observed, but the emission intensity decreased systematically. In a separate experiment, aqueous NaOH was added gradually to reserpine solution up to the permissible limit, as mentioned in the absorption experiment, and the fluorescence intensity was found to increase systematically without any change in the emission peak. However, the rate of change of the fluorescence intensity was less prominent in the case of base addition than that in acid addition. All of the emission spectra were merged together and are shown in Figure 9. The dotted curve in this figure represents the fluorescence spectrum of reserpine in aqueous solution (≈10^{-6} M). The fluorescence intensity increases from the lowermost to the uppermost value for a change in pH from 1.5 to 10.5. Principle and working formulas analogous to those in the absorption experiment were used. The linear plots with fluorimetric data at the emission peak in both the acidic and alkaline ranges are shown in Figures S5 and S6 of the Supporting Information. The values of $(\text{p}K_a)_1$ and $(\text{p}K_a)_2$ from such plots are, respectively, 4.10 ± 0.02 and 9.44 ± 0.02, which are in close agreement with the values obtained in the absorption spectrometric method.

3.6. Fluorimetric Determination of the Formation Constant of the Reserpine−*o*-Chloranil CT Complex. Addition of *o*-chloranil solution to reserpine systematically decreases the fluorescence intensity of the latter, as shown in Figure 10. When the emission intensity data (Table 5) were analyzed according to the simple Stern−Volmer equation, deviation in the upward direction was observed, suggesting the formation of a ground-

state complex (Figure S7 of the Supporting Information). The data were then subjected to the modified Stern−Volmer equation[23]

$$\frac{I_f^0 - I_f}{I_f[Q]} = (K_{SV} + K) + K_{SV}K[Q] \quad (20)$$

where I_f^0 is the intensity of fluorescence in the absence of quencher (here, *o*-chloranil), I_f is that in prence of quencher, and [Q] is the quencher concentration. The constants K_{SV} and K are, respectively, the Stern−Volmer constant and the formation constant of the CT complex. A very good linear plot with $r^2 = 0.99$ was obtained as required by eq 20 (shown in Figure S8 of the Supporting Information). From the slope and intercept of this plot, K was found to be $(1.49 \pm 0.02) \times 10^4$ dm^3 mol^{-1}, which, considering the order of magnitude, is close to the value obtained from the absorption experiment.

4. Conclusion

In the present study, the individual proton affinities $(pK_a)_1$ and $(pK_a)_2$ of the N atom of reserpine have been determined spectrophotometrically in two independent ways by studying the spectral variation of (a) reserpine and (b) its charge-transfer complex with changes in pH. The small difference in $(pK_a)_1$ obtained by the two methods may be attributed to the fact that the quinone-type acceptor may be protonated to some extent under the experimental conditions, depending on its proton affinity. In the present case, DDQ is not a suitable acceptor for this reason. However, *o*-chloranil gives a fairly reliable result. The pK_a determination using *p*-chloranil was not performed because the CT peaks appear too far away from the visible range (281 nm). The results obtained were further checked by noting the variation of the fluorescence intensity of reserpine upon addition of *o*-chloranil, acid, and base, and almost complete agreement with the absorption spectrometric result was observed.

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Supporting Information Available: Additional experimental information and figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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