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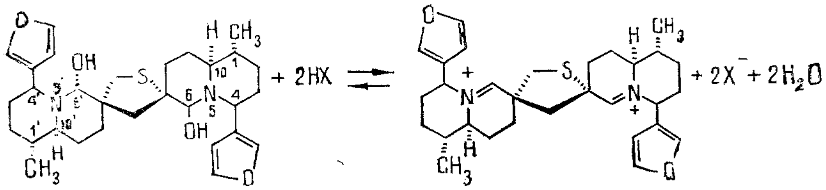
IONIZATION CONSTANTS OF A NUMBER OF ALKALOIDS USED IN MEDICINE

M. E. Perel'son, I. V. Persiyanova,
T. S. Semenova, and I. E. Kopylova

UDC 541.127+537.57+547.94+615.322

The pK_a values of a number of alkaloids in water or in aqueous ethanol have been measured by the potentiometric titration method: nuphleine — 4.59, 6.98; brevicolline — 5.17, 8.02; chelidonine — 6.40; sanguinarine — 7.32; chelerythrine — 7.53; stepharine — 8.48; d-pseudoephedrine — 9.49. For nuphleine and brevicolline, each of which has two nitrogen atoms capable of protonation, assignments have been made of pK_a values to the corresponding atoms on the basis of the results of UV spectroscopy.

Information on the extent to which substances are ionized at a particular pH value is necessary both for choosing the optimum technology of isolating alkaloids from plant sources and also for the correct determination of the quality indices of the substances of the preparations and their medicinal forms.



We have determined the pK_a values of a number of alkaloids used in medical practice as drugs or present in plants used as drugs: d-pseudoephedrine, stepharine, brevicolline, nuphleine, sanguinarine, chelerythrine, and chelidonine (Table 1). All the alkaloids investigated are bases of medium strength in water or aqueous ethanol and their ionization constants differ by several orders of magnitude.

The nuphleine and brevicolline molecules each have more than one nitrogen atom, and therefore we shall consider the features of their ionization. Salt formation by nuphleine, as an α-carbinolamine, is accompanied by the splitting out of two molecules of water and the appearance of two $C{=}\overset{+}{N}$ double bonds [1]. This is confirmed, in particular, by the presence in the PMR spectrum of nuphleine in trifluoroacetic acid of two one-proton singlets at 7.96 and 8.27 ppm (0 — TMS) due to two $HC{=}\overset{+}{N}$ groups, and an absorption band at 293 nm in the UV spectrum (96% ethanol) of a salt of hydrochloric acid.

The fact that two nitrogen atoms separated by five saturated carbon atoms have ionization constants differing by more than two orders of magnitude indicates their different basicities. The question of which of the nitrogen atoms is the more basic (pK_a 6.98) was answered with the aid of UV spectroscopy.

It has been shown [2] that absorption in the 290-295-nm region in acid solutions of thiospiran alkaloids is connected with an intermolecular interaction of the sulfur atom and

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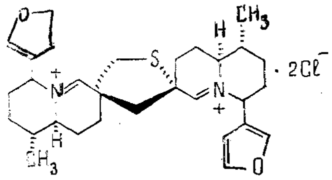
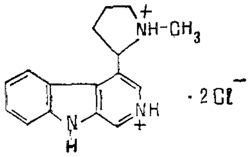
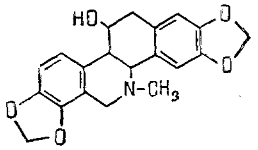
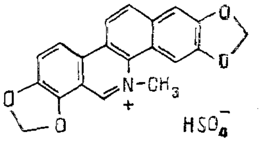
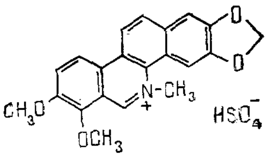
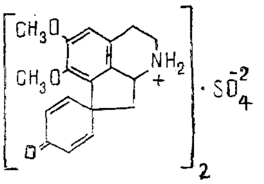
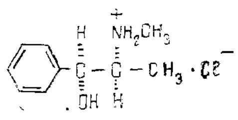
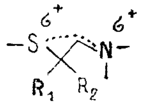
TABLE 1

Compound	Structural formula	Solvent	pK_a
Nuphleine bisanhydrodichloride		50% ethanol	4,59±0,05 6,98±0,05
Brevicolline dihydrochloride		Water	5,17±0,04 8,02±0,05
Chelidonine		50% ethanol	6,40±0,05
Sanguinarine bisulfate		Water	7,32±0,07
Chelerythrine disulfate		Water	7,53±0,06
Stepharine sulfate		Water	8,48±0,06
d-Pseudoephedrine hydrochloride		Water	9,49±0,05



an immonium ion as the result of the inclusion of the sulfur atom in a three-membered ring with the formation of the grouping.

As has been shown [2], a double bond in the 5',6' position gives absorption in the short-wave region. Thus, it is possible to judge the degree of ionization of the nitrogen atom in position 5 from the intensity of the band at 293 nm in the UV spectrum.

TABLE 2. Characteristics of the UV Spectra of Nuphleine at Various pH Values (50% Ethanol)

pH	α, %*		$\left[E^{1\%}_{1\,cm}\right]^{293}$†	$\frac{\left[E^{1\%}_{1\,cm}\right]^{293}\cdot 100}{35,6}$
	pKa 4,59	pKa 6,98		
2,75	98,6	100,0	35,6	100 0
3,35	94,6	100,0	35,6	100,0
5,36	14,52	97,3	33,3	93,5
6,80	0 61	56,3	19,8	55,6

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*Percentage of the ionized form.

†Taking into account the absorption due to the wing of the short-wave band due to the second $C=\overset{+}{N}$ bond ($\lambda_{max} < 220$ nm).

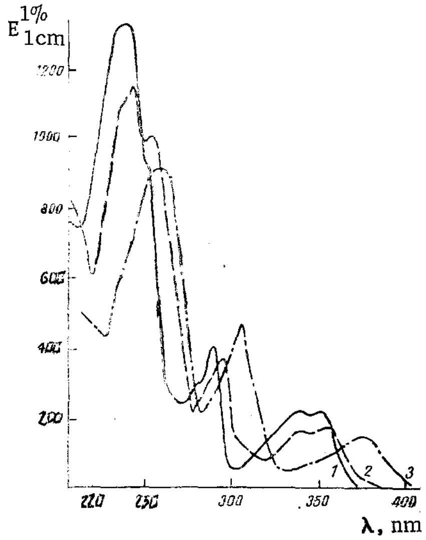

Fig. 1. UV spectrum of brevicolline hydrochloride in water at the following pH values: 1) 10.4; 2) 6.28; 3) 2.14.

The results of a study of the UV spectra of nuphleine in 50% ethanol at various pH values are given in Table 2. The change in the specific absorption index of nuphleine as a function of the pH of the solution corresponds completely to the change in the percentage in the amount of the ionized form of the more basic nitrogen atom (pK_a 6.98). Consequently, the pK_a value of 6.98 corresponds to the ionization of the nitrogen atom in position 5 and the value of 4.59 to that in position 5'.

The brevicolline molecule contains three nitrogen atoms: pyrrole, pyridine, and pyrrolidine nitrogens. Because of the inclusion of its unshared pair of electrons in a heteroaromatic ring, the pyrrole nitrogen atom is protonated with great difficulty; pK_a for pyrrole is −0.27 [3].

The pK_a values determined relate to the pyridine and pyrrolidine nitrogen atoms, and their assignment can be made on the basis of literature information concerning the pK_a values of compounds with similar structures: 11.27 for pyrrolidine and 5.17 for pyridine [3]. A simple comparison shows that a pK_a of 5.19 most probably relates to the pyridine nitrogen atom and a pK_a value of 7.99 to the pyrrolidine nitrogen atom. This conclusion is confirmed by a consideration of the UV spectra of brevicolline in water at various pH values (Fig. 1). It must be expected that the protonation of the pyrrolidine pyridine nitrogen atom should lead to smaller changes in the UV spectrum than that of the pyridine nitrogen atom. At pH 10.16, more than 99% of the alkaloid is present in the form of a base while at pH 2.14 practically all the molecules exist in the diprotonated form. At pH 6.28, more than 98% of the molecules are protonated at one nitrogen atom and about 8% at two.

It can be seen from Fig. 1 that the UV spectrum at pH 6.8 is closer to the spectrum of the base than to the spectrum of the diprotonated alkaloid, which permits a qualitative conclusion concerning the initial ionization of the pyrrolidine nitrogen atom. This conclusion cannot be confirmed quantitatively because of the impossibility of obtaining the pure spectrum of the monoprotonated form since at pH values between 2 and 10 the substance exists in at least two forms.

EXPERIMENTAL

The ionization constants were determined from the curves of the potentiometric titration of 0.001 M solutions of the alkaloids or their salts in water or in 50% ethanol. Calculation was performed for eight points, corresponding to 10-80% neutralization. The titration was carried out not less than three times. Table 1 gives the mean pK_a values and their standard deviations. Titration was performed with glass (G 2222B) and calomel electrodes on a PHM-26 pH meter (Radiometer, Denmark) at 25 ± 1°C.

The substances studied were kindly provided by L. D. Yakhontova, I. I. Fadeeva, N. M. Margvelashvili, and O. E. Lasskya.

Nuphleine Bisanhydrodichloride. $[\alpha]_D^{20}$ + 225° (c 1.5; water). When 100 μg of the substance was chromatographed in a thin layer of alkaline silica gel in the chloroform—methanol (20:0.5) system and the chromatogram was treated with iodine vapor, no spots of other substances were detected (threshold sensitivity of nuphleine 1 μg).

Brevicolline Dihydrochloride, mp 272°C. When 100 μg of the substance was chromatographed in a thin layer of alkaline silica gel in the chloroform—methanol (9:1) system and the chromatogram was treated with iodine vapor, only one spot of brevicolline was detected (threshold sensitivity for brevicolline about 0.5 μg).

Free Chelidonine. mp 132-133°C, $[\alpha]_D^{20}$ + 105° (c 2.09; chloroform). When 100 μg of chelidonine was chromatographed in a thin layer of alkaline silica gel in the chloroform—toluene—methanol—ethyl acetate (40:20:5:15) system and the chromatogram was treated with iodine vapor, only one spot was detected (threshold sensitivity for chelidonine 1 μg).

Sanguinarine Bisulfate and Chelerythrine Bisulfate. Chromatographically pure samples were used; when 200 μg was deposited and chromatographed in a fixed layer of silica gel in the petroleum ether—diethyl ether—methanol (15:35:3) system, no spots of other alkaloids were detected (threshold sensitivity for sanguinarine and chelerythrine 0.1 μg). PMR spectra in trifluoroacetic acid confirmed the authenticity of the sanguinarine and chelerythrine bisulfates and showed the absence from the samples used of other compounds containing hydrogen atoms.

Stepharine Sulfate. mp 250-251°C, $[\alpha]_D^{20}$ + 121.2α (c 0.87; water). When 250 μg of the substance was chromatographed on a thin layer of nonfixed alkaline silica gel in the ethyl acetate—chloroform—methanol (2:1:3) system and the chromatogram was treated with the Dragendorff reagent, no other alkaloids were detected.

d-Pseudoephedrine Hydrochloride. mp 184-185°C, $[\alpha]_D^{20}$ + 62.2° (c 5; water). The PMR spectrum in trifluoroacetic acid confirmed the authenticity of the sample and showed the absence of impurities containing hydrogen atoms.

CONCLUSION

The pK_a values in water or aqueous ethanol have been measured by potentiometric titration for a number of alkaloids: nuphleine, brevicolline, chelidonine, sanguinarine, chelerythrine, stepharine, and d-pseudophedrine. For nephleine and brevicolline, each of which has two nitrogen atoms capable of protonation, pK_a values have been assigned to the corresponding atoms on the basis of the results of UV spectroscopy.

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