In particular the ^{13}C NMR measurements of Ms. A. D. Kahle, the mass spectral determination of Dr. R. A. Coombs, and the x-ray work of Dr. H. P. Weber, Sandoz, Basle, are appreciated.

Registry No.—**1**, 6642-31-5; **2**, 6135-14-4; **3**, 1186-70-5; **4a**, 60581-88-6; **5a**, 60581-89-7; **6f**, 58-55-9; **10**, 10513-47-0; **11a**, 60581-90-0; **12a**, 60581-91-1; **12c**, 58253-99-9; **12d**, 60581-92-2; **13a**, 60581-93-3; pinacolone, 75-97-8; 6-amino-1,3-dimethyluracil, 6642-31-5; ClSO$_2$NCO, 1189-71-5; *N*-*tert*-butyl-5-methylisooxazolinium perchlorale, 60581-94-4.

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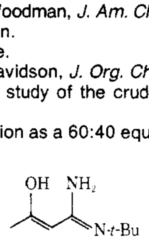
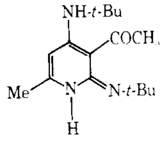
(26) Amidine **13a** exists in solution as a 60:40 equilibrium mixture of **13a** and tautomer i.



i

(27) An isomeric structure, such as ii, cannot be ruled out on the basis of our analytical data.



ii

Determination of Ionization Constants of Alkaloids by Paper Electrophoresis

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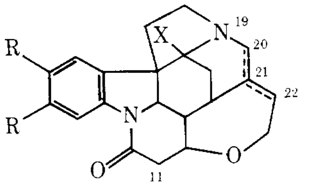
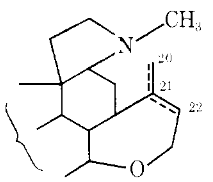
The ionization constants in water of strychnine, brucine, and seven related compounds have been determined by paper electrophoresis using microgram quantities of the bases. Results are in fair agreement with values obtained by potentiometric titration or by changes in solubility as a function of pH.

Arguments based on p*K* values are often useful in establishing the structures of alkaloids and other natural products.[1] A very simple method for determining p*K* by paper electrophoresis has been described,[2] but was tested only with fairly simple compounds, all reasonably soluble in water. In this paper we examine the usefulness of this method for strychnine (**1**), brucine (**2**), and several related compounds of very limited solubility in water. For such compounds the temptation to determine p*K* values in mixed aqueous organic solvents is very great but, according to Albert and Serjeant, "should be resisted",[3] because otherwise one loses the advantages accruing from the immense amount of data in the literature for purely aqueous solutions. To check the values obtained by paper electrophoresis, we have also determined the p*K* values of the bases **1**–**5** and **7**–**9** by a solubility method[3] which takes advantage of the limited water solubility of these compounds. The *N*-oxide **6** was sufficiently soluble in water for the conventional potentiometric titration procedure to be used.





1, strychnine: R = X = H; Δ^{21}
2, brucine: R = OMe; X = H; Δ^{21}
3, neostrychnine: R = X = H; Δ^{20}
4, neobrucine: R = OMe; X = H; Δ^{20}
5, pseudostrychnine: R = H; X = OH; Δ^{21}
6, strychnine *N*-oxide: $\geq\overset{+}{N}{}^{19}\!-\!\overset{-}{O}$ in place of $\geq N^{19}$
7, benzylidene strychnine: $>C^{11}\!=\!CHPh$ in place of $>C^{11}H_2$
8, base C, Δ^{20}, R = H
9, base D, Δ^{21}, R = H

Table I. Adsorptive Factors of Protonated (ρ_{BH^+}) and Free Bases (ρ_B), and Relative Mobilities (u_r) and Ionization Constants (p*K*) of Protonated Bases 1–9 in Water

				u_r		p*K*	
Registry no.	Base	ρ_{BH^+}[a]	ρ_B[b]	Calcd	Exptl	PE[c]	Solubility[d]
57-24-9	**1**	0.73	0.64	0.55	0.56	8.4	8.41 ± 0.07[f]
357-57-3	**2**	0.66	0.53	0.48	0.46	8.2	8.41 ± 0.05[g]
466-69-3	**3**	0.70	0.63	0.53	0.51	4.5	4.44 ± 0.06
60606-95-3	**4**	0.66	0.58	0.48	0.45	4.5	4.57 ± 0.04
465-62-3	**5**	0.67	0.62	0.51	0.55	6.1	6.13 ± 0.05
7248-28-4	**6**	0.78	0.73	0.58	0.58	3.8	4.13 ± 0.02[e]
25998-70-3	**7**	0.36	0.09	0.24	0.25	*h*	8.38 ± 0.08
14320-69-5	**8**	0.76	0.57	0.56	0.55	7.6	7.54 ± 0.10
30291-05-5	**9**	0.76	0.56	0.56	0.56	7.7	7.24 ± 0.06

[a] From R_f measurements at pH 2.1. [b] From R_f measurements at pH 10.2. [c] By paper electrophoresis. [d] By solubility measurements, except for *e*. [e] Potentiometric titration used. [f] Everett et al.[4] report 8.26 at 25 °C, from titration in dilute solution. [g] Cage[5] reports 8.28 at 25 °C, from titration. [h] Excessive streaking of spots on paper.

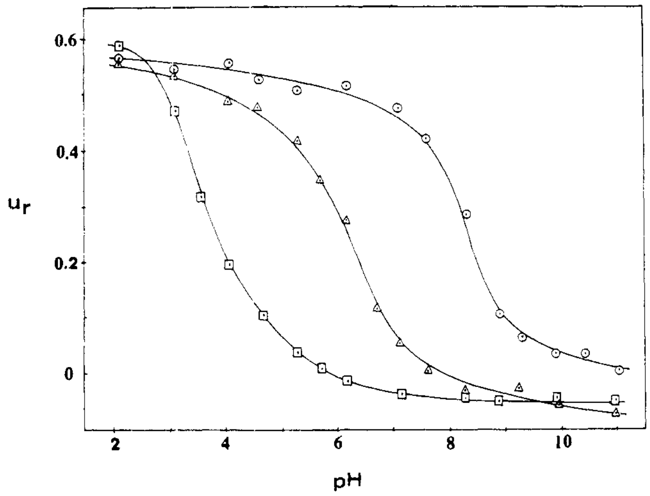


Figure 1. Effect of pH on relative mobilities u_r of strychnine (○), pseudostrychnine (△), and strychnine *N*-oxide (□).

Results and Discussion

The p*K* values of **1–5** and **7–9** were determined by following the change of solubility with pH, using ultraviolet absorption as a convenient measure of solubility. These values (Table I) agree fairly well with p*K* values obtained by potentiometric titration of very dilute solutions, where comparison is possible.[4,5]

The solubility method requires fairly large amounts of material, in contrast to the method of paper electrophoresis, which can be done with less than 100 μg of material if necessary. In the latter method, relative mobilities u_r of the alkaloidal base are plotted against pH. Sigmoid curves are obtained, as shown in Figure 1, and the pH at the inflexion point of the sigmoid curve (found by the graphical method of Stewart and Yates[6]) gives the p*K* of the protonated base. The p*K* values thus obtained prove to be in fair agreement with those obtained by the more reliable solubility method, as shown by the comparison in Table I. It should be noted that the p*K* values obtained by paper electrophoresis are for aqueous solutions at 28.5–30.0 °C (as measured by a thermocouple in contact with the moist paper), depending on the current, and so exact agreement cannot be expected.

The p*K* values thus determined for compounds **1**, **2**, **3**, and **5**, all tertiary amines, show a linear relationship with the pK^*_{DMC} values for these compounds in 80% dimethyl Cellosolve:[7]

$$pK^*_{DMC} = 0.82\,pK + 0.41 \tag{1}$$

On the other hand, the *N*-oxide **6**, a different type of base, does not obey this relation, nor do the ephedrine-type bases studied by Prelog and Häfliger.[8]

The p*K* value of strychnine shows it to be about 500 times less basic in water than the bridged tertiary amine quinuclidine;[1] part of this difference may be due to the inductive or field effects of ether and amide groups, part to ring strain.[1] The further decrease in basicity in neostrychnine (**3**) and neobrucine (**4**) has been attributed to increased strain.[1] One might expect then, on naive grounds, an increased basicity for **8** and **9** because of relief of strain.[1] However, models show the protonation (let alone the solvation) of the tertiary amino groups of these compounds to be severely hindered, and they prove to be less basic.

Theoretical Considerations. Some appreciation of the underlying theory[9,10] is desirable if one is devising a procedure for a novel set of acids or bases. Thus for reasonable accuracy the standard ion should be chosen to give $u_r \simeq 1$ when a base is completely protonated. For approximately spherical ions (as in the present case), and in an apparatus where evaporation from the paper is prevented, u_r is given by[9,10]

$$u_r = u/u_{std} = (v_{w(std)}/v_w)^{1/3}(\rho z/\rho_{std}z_{std}) \tag{2}$$

where u and u_{std} are the mobilities of the variably protonated base and of the standard ion, v_w and $v_{w(std)}$ are van der Waals volumes,[11] ρ and ρ_{std} are adsorptive factors measuring retardation by reversible adsorption onto the paper,[10] and z and z_{std} are the charges on the ions.

At pH 2, $z \simeq z_{std} = +1$, $\rho_{std} \simeq 1$, and ρ_{BH^+} (Table I) can be equated (roughly) with the R_f value obtained by ascending chromatography on paper strips. However, in order to obtain the calculated u_r values of the protonated bases BH$^+$ of Table I, it has been necessary to use the empirical equation

$$u_r = 0.9\rho_{BH^+}(v_{w(std)}/v_w)^{1/3} \tag{3}$$

This may be due to slightly different numerical factors in the Stokes equation for the migration of the protonated alkaloid and the standard ion, because of their very different sizes.[11]

As the pH increases, BH$^+$ is progressively converted into B, and u_r decreases because of (a) a drop in the net charge z (z_{std} remaining +1)

$$z/z_{std} = [BH^+]/([BH^+] + [B]) = [H^+]/([H^+] + K) \tag{4}$$

and (b) a drop in ρ:

$$\rho = \frac{\rho_{BH^+}[H^+] + \rho_B K}{[H^+] + K} \tag{5}$$

The overall dependence of u_r on pH is given by

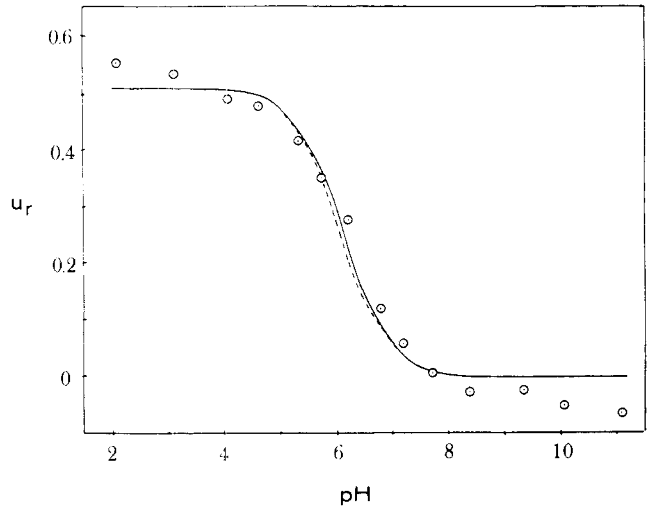


Figure 2. Effect of pH on relative mobility of pseudostrychnine. Points: experimental; broken curve, theoretical for eq 6 with pK = 6.1 and ρ_B, ρ_{BH^+} from Table I; solid curve, theoretical for eq 7.

$$u_r = 0.9\left(\frac{v_{w(std)}}{v_w}\right)^{1/3}\left(\frac{[H^+]}{[H^+]+K}\right)\left(\frac{\rho_{BH^+}[H^+]+\rho_B K}{[H^+]+K}\right) \qquad (6)$$

In practice, the change from ρ_{BH^+} to ρ_B is small enough so that it can be ignored, and the last term replaced by ρ_{BH^+}:

$$u_r = 0.9\left(\frac{v_{w(std)}}{v_w}\right)^{1/3}\left(\frac{[H^+]}{[H^+]+K}\right)\rho_{BH^+} \qquad (7)$$

This is shown by a comparison of the theoretical curves according to eq 6 and 7 in Figure 2. The agreement of these curves with the experimental points is only approximate, as should be expected in our relatively simple apparatus in which evaporation from the paper is not prevented,[12] and thus the pK values are not as accurate as might be desired. This problem may be overcome by the use of more precisely controlled equipment.

Experimental Section

Materials. Strychnine and brucine were commercial products. Neostrychnine,[13] neobrucine,[13] pseudostrychnine,[14] strychnine N-oxide trihydrate,[14] benzylidene strychnine,[15] base C,[16] and base D[16] were prepared according to the literature. A 1% solution of allyltriethylammonium bromide was prepared by reaction of allyl bromide with triethylamine in ethanol-water (4:1); it was not necessary to isolate the salt.

Buffer solutions of ionic strength $\mu \simeq 0.01$ covering the pH range 2–11 were prepared from standard solutions of hydrochloric acid, potassium chloride, potassium acid phthalate, potassium dihydrogen phosphate, sodium hydroxide, and sodium borate.

Paper Electrophoretic Procedure. Strips of Whatman 3 MM chromatography paper 30 cm long and 3 cm wide were placed in the rack of a Beckman Spinco electrophoresis cell (Durrum type) and wetted with buffer solution. After 30 min equilibration in the cell, 10 μl of 1% ethanolic solutions of the following three compounds were spotted on a line pencilled across the midpoint of the strip: the base being investigated; acrylamide, a neutral marker of bulk flow of the buffer solution because of electroendosmosis, evaporation, etc.; and allyltriethylammonium bromide, a standard ion of constant charge +1. A current of 12 mA was passed through the strips for 30 min. They were then removed, partially dried, and sprayed with an acetone solution of potassium permanganate, which revealed transitory yellow spots on purple background (a general test for compounds containing an olefinic double bond). Brucine compounds could also be visualized with a spray of concentrated nitric acid, which showed orange spots on a white background. The spots were outlined with pencil before they faded, and distances measured from the center of the acrylamide spot to the center of the allyltriethylammonium spot (d_{std}), and to the center of the alkaloid spot (d). The relative mobility u_r of the alkaloid is then given by $u_r = d/d_{std}$. Measurements in triplicate were averaged.

Determination of Ionization Constants by Solubility Measurements. The approximate pK of the base was obtained by paper electrophoresis (vide supra). Saturated solutions of the base in several buffer solutions ($\mu \simeq 0.01$) having pH values in the range pH = pK ± 0.8 were then prepared by shaking for 24 h an excess of the solid with 10 ml of buffer solution, kept at 25.0 °C by a thermostat bath. The suspensions were centrifuged, and the pH and absorbance A (at 300 nm for compounds **2**, **4** and **7**, and at 255 nm for all other compounds) of the supernatant solutions were determined. At the same time the absorbance A_B of a solution[17] saturated only with the basic form B of the alkaloid was obtained at a pH = pK + 3. The apparent ionization constant pK' of the conjugate acid BH$^+$ was obtained as the intercept of the plot of log $[(A/A_B) - 1]$ against −pH, following the equation

$$\log\,[(A/A_B) - 1] = -\text{pH} + \text{p}K' \qquad (8)$$

In water at 25 °C the apparent ionization constant pK' is related to the thermodynamic constant[3] pK by

$$\text{p}K = \text{p}K' - 0.512\sqrt{\mu}/(1 + 1.6\sqrt{\mu}) \qquad (9)$$

The difference indicated by the last term in this equation is 0.04, which is within the experimental error of our measurements, and can be neglected.

Acknowledgment. The financial support of the National Research Council of Canada is gratefully acknowledged.

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