Physical Chemistry of Ergot Alkaloids and Derivatives I: Ionization Constants of Several Medicinally Active Bases

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Abstract □ A novel procedure is presented for determination of the ionization constants of several pharmacologically active naturally occurring ergot alkaloids and their congeners. For this purpose use was made of the complexation and consequent solubilization of these poorly soluble molecules by certain xanthines with special emphasis on 7-β-hydroxypropyltheophylline. This method allows an approach to investigation of the approximate pKa values of the proteinaceous alkaloids such as ergotamine which resisted the usual means available for measurement of dissociation constants. It is also illustrative of the weak attractive forces present in complexes of this nature.

Keyphrases □ Ergot alkaloids, congeners—ionization constants, determination □ Dissociation-constant determination—methodology □ Titration, aqueous—dissociation-constant determination

Difficulties were experienced on attempted evaluation of the ionization constants for various ergot alkaloids and their analogs. The insolubility of the bases in aqueous media has been discussed previously by the authors (1–3). This limitation is likewise encountered in ethanol–water solutions sometimes employed with apparent success in pKa measurements (4). The alkaloidal base, ergotamine, exhibits little solubility in polar solvents such as ethanol and propylene glycol, and this is the situation with many related compounds. Ergotamine and other ergot alkaloids possess an UV spectrum but only small differences are discernible between the ionized species at pH 2 and the neutral species at pH 11, thus precluding this route for analysis of the ionization constants. Somewhat erratic solubility properties have been noted in this laboratory for ergot alkaloids and derivatives when the cyclic tripeptide moiety is intact, leading to a lack of precision and confidence in a pKa obtained by this technique.

Preliminary investigations and previous communications (1–3) indicated an elevation in solubility of the neutral molecule as well as prevention of its precipitation upon inclusion of certain xanthines. A study was undertaken to detect if this phenomenon would have applicability in measurement of ionization constants of these relatively complex molecules.

EXPERIMENTAL

Dissociation constants were determined by titration of the hydrochlorides or methanesulfonates of the bases in aqueous solution (0.005 mole of the methanesulfonate salt or 0.005 mole of the base solubilized by 5 ml. 0.1 *N* HCl in 92.5 ml. freshly boiled water) along with 10–20 g. 7-β-hydroxypropyltheophylline (see Table I). This solution was titrated with 10 equal increments (0.5 ml.) of carbonate-free 0.1 *N* KOH. The pH was taken initially, following each portion of KOH titrant, and nine values for the ionization constant calculated in each case (5).

The same procedure was carried out with the two relatively soluble bases, methysergide and methylergonovine, except the xanthine was omitted.

Table I—Ionization Constants of Ergot Alkaloids and Derivatives[a]

Compound	% Xanthine[b]	pKa
1. Methysergide	0	6.62 (± 0.02)[c]
2. Methylergonovine	0	6.65 (± 0.03)[c]
3. Dihydroergocristine	10	6.74 (± 0.02)[d, e]
4. Dihydroergocriptine	10	6.74 (± 0.02)[d, e]
5. Dihydroergocornine	10	6.76 (± 0.02)[d, e]
6. Dihydroergotamine	10	6.75 (± 0.03)[d, e]
7. Ergostine[f]	15–20	6.30 (± 0.04)[d, e]
8. Ergotamine[f]	15–20	6.25 (± 0.04)[d, e]
9. Ergotaminine[f]	15–20	6.72 (± 0.04)[d, e]

[a] All measurements at 24°. [b] 7-β-Hydroxypropyltheophylline. [c] Thermodynamic ionization constant. [d] Measured pKa values in presence of corresponding xanthine percentage (Column 2). [e] Corrected pKa values for Compounds 3–9 are 6.89 (±0.07), 6.89 (±0.07), 6.91 (±0.07), 6.90 (±0.08), 6.45 (±0.09), 6.40 (±0.09), and 6.87 (±0.09), respectively, and are calculated by addition of 0.15 (±0.05) to the measured values (Column 3) and should closely approach the thermodynamic pKa. See under *Results and Discussion*. [f] Excess HCl added for dissolution of base followed by neutralization with KOH.

MATERIALS

Ergotamine base was twice recrystallized from acetone–water (90 ml. acetone: 10 ml. water); methysergide base was recrystallized four times from methanol–water (10 ml. methanol: 40 ml. water), followed by drying at 50° (1 mm.) for 24 hr.

Other alkaloidal bases and methanesulfonate salts were chromatographically pure (traces of contaminants) and were dried overnight at 50° (1 mm.).

7-β-Hydroxypropyltheophylline, m.p. 135–138° (Ganes Chemical Works, Inc., New York) was used.

Tris(hydroxymethyl)aminomethane (THAM) (primary standard), pKa = 8.18 at 20° (Fisher Sci. Co., Fairlawn, N. J.) was used.

The pH values were measured on a Metrohm pH meter using electrodes[1] standardized on 0.05 *M* potassium hydrogen phthalate (pH 4.0, 24°) and 0.05 *M* sodium borate (pH 9.20, 24°). All measurements were made at 24°.

RESULTS AND DISCUSSION

Utilization of mixed solvent systems in evaluation of dissociation constants for substances showing poor aqueous solubility has been made rather frequently since the procedure was first reported (6). Although this expediency should probably be avoided whenever

[1] Corning triple-purpose glass electrode No. 476020 and calomel electrode No. 476002, Corning Glass Works, Medfield, Mass.

possible (5), there are circumstances where it must be resorted to for obtaining anything resembling satisfactory pKa values.

It has been seen in this laboratory that ergotamine and other alkaloids of ergot having a cyclic tripeptide attached to the lysergic or isolysergic portions of the molecule generally exhibit low solubility not only in water but in many water-miscible solvents. This fact tends to complicate titrimetric analysis. Fortunately the ionization constants of protonated amines, such as dihydroergocristine methanesulfonate, are frequently little different in mixed solvents than in water alone.

Neutral xanthines possess the ability to solubilize the free alkaloids to some extent (1), and it was hoped by this means titration could be carried out. The xanthine, 7-β-hydroxypropyltheophylline, was chosen for extensive work because of its high water solubility relative to caffeine, theophylline, and others. The results of the study are listed in Table I.

The compounds in Table I have three or more nitrogen atoms present in their structures; however, only the ring nitrogen located in the tetrahydropyridine or hexahydropyridine moieties of the lysergic and isolysergic acid portions (7–9) need be considered in this work. The indole nitrogen (amine) in the lysergic or isolysergic acid fragment is weakly basic and of no consequence in normal pH ranges (7). The amide nitrogen present in methysergide and methylergonovine (Table I, Compounds 1 and 2) displays insignificant pH effect over the interval investigated, and this holds for the additional peptide nitrogens of the more complex molecules (Table I, Compounds 3–9).

The pKa values were calculated using the expression

$$pKa = pH + \log \frac{BH^+}{B} \qquad \text{(Eq. 1)}$$

where BH^+ = alkaloidal salt concentration (HCl or methanesulfonate) and B = alkaloidal base concentration. No correction was necessary for OH^- or H^+ ions in the range of pH's studied. KOH titrant was added in 10 equal increments and the pKa value obtained for each of the nine points (5). Results with a precision of less than ±0.04 were rejected.

A slight perturbation of the ionization constant was evidenced with the more soluble compounds in Table I, methysergide and methylergonovine, on inclusion of xanthine. Since these materials have the capacity to complex as well as ergotamine and the other ergot derivatives studied (1–3), this parameter is present in all compounds under consideration. Measured pKa values for these two substances declined upon introduction of 7-β-hydroxypropyltheophylline in quantities of 2 to 30 g./100 ml. solvent. There was little change in the measured pKa (Table I, Column 3) following this initial drop of 0.1–0.2 units, 0.15 (±0.05),[2] on addition of xanthine. Extrapolations of the data as a plot of pKa *versus* percent xanthine would certainly produce erroneous values. This may be seen in Fig. 1 in the case of methysergide, where extrapolation would lead to a pKa somewhat lower than it actually is. It may be noted in Fig. 1 that there appears this small initial alteration of the ionization constant on addition of the xanthine followed by a degree of leveling.

In some of the proteinaceous alkaloids, such as dihydroergocristine and dihydroergocriptine, the end values could be obtained for the previously stated equation before precipitation of the free base took place, where BH^+/B = 39/1 to 9/1. In these instances the dissociation constants derived from these calculations were found to be greater by the previously stated increment, 0.15 (±0.05)[2] than the observed pKa when sufficient xanthine was present to solubilize the alkaloid. The quantities of 7-β-hydroxypropyltheophylline used (Table I, Column 2) were those experimentally found to hold the base in solution. This lowering of the ionization constant occurred with THAM and remains fairly constant over a wide proportion of xanthine levels. Caffeine, theophylline, and other analogs give satisfactory results for many of these ergot derivatives when employed for pKa determination.

[2] The value, 0.15 (±0.05), does not refer to the precision of the experimental results but to a lowering of the pKa value by 0.1 to 0.2 on addition of xanthine. The precision of all values obtained with xanthine are listed in Column 2, Table I.

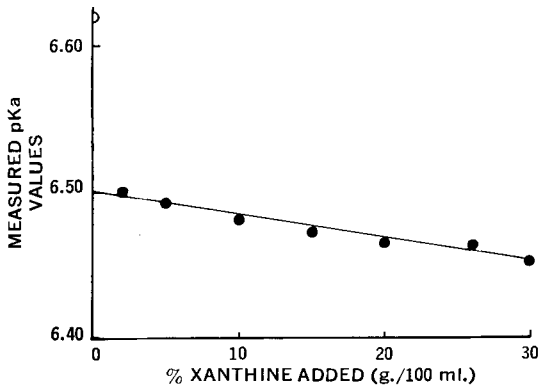

Figure 1—*Plot of −log Ka (pKa) measured for methysergide (0.01 mole of HCl salt in 100 ml. water; precision of experimental results not indicated but greater than ±0.03 in every case) against the concentration of 7-β-hydroxypropyltheophylline (g./100 ml.). Key: ○, pKa at zero xanthine concentration—thermodynamic pKa; and ●, measured pKa in presence of amount of xanthine indicated on abscissa.*

The numbers in Table I (superscript ^{e}) are the corrected pKa values for Compounds 3–9 and are the best obtainable from available data. Each of these values is derived by addition of this factor[2] to the measured pKa (Column 3), excepting Compounds 1 and 2 where the thermodynamic ionization constant could be found by titration. Although no claim is made regarding their exactness, these values should closely approach the thermodynamic ionization constant.

This method offers a serviceable approach to determination of the ionization constants of the ergot alkaloids and derivatives of proteinaceous nature. It is extremely reproducible and easy to apply. The small effects demonstrated by rather large quantities of complexing agent on the pKa give more circumstantial evidence for the weak attractive forces present between the two types of molecules.

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