

ABSORPTION OF PYRIMIDINES, PURINES AND NUCLEOSIDES BY Na-, Mg-, AND Cu (II)-ILLITE.
(CLAY-ORGANIC STUDIES. XVI)

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ABSTRACT

Absorptions of various purines, pyrimidines and nucleosides from aqueous solutions by Na-, Mg-, and Cu(II)-saturated illite have been measured in the pH range 2-10. Illite from Skytop, Pennsylvania, was treated to remove organic and Fe-hydroxy contaminants and a <1 micron fraction was separated for the absorption measurements. The cation exchange capacity of the <1 micron fraction was 0.14 ± 0.01 meq/g illite and the surface area measured by krypton absorption was 65 m²/g.

With Na- and Mg-saturated illite, organic absorption occurs mainly under acid conditions by a cation exchange mechanism. The pH of the equilibrating solution for maximum absorption shows a linear relationship to pK_a for the formation of protonated molecules. The maximum absorption of different molecules diminishes as pK_a diminishes and as more acid conditions are required for protonation. With Cu(II)-saturated illite, a cation exchange process operates at low pH values; at higher pH values, about 6.5-8, complex formation occurs between the organic molecules and the Cu(II) ions, and the percentage absorption is related to the electron releasing ability of the substituent groups attached to the purine and pyrimidine bases.

INTRODUCTION

Very few studies have been made of organic absorption by illites compared with the many studies of organic absorption by montmorillonites. The relatively small specific surface area of illite, its small cation exchange capacity, and the inapplicability of x-ray diffraction methods are obvious disadvantages when studying organic absorption by illite. Nevertheless there are cogent reasons why absorption by illite should be considered, namely: (1) The prevalence of illite rather than of montmorillonite in many sediments and argillaceous rocks; (2) The fact that when external surfaces only are involved, restrictions on the absorption process arising from the bulky forms of some molecules are likely to be less important than when these molecules must penetrate (or diffuse) into the interlayer positions of montmorillonite. Grim, Allaway and Cuthbert (1947) showed that under acidic conditions, protonated organic molecules (cations) can participate in exchange reactions with the exchangeable inorganic cations of illite and that the total absorption may exceed the exchange capacity due to additional absorption of neutral molecules.

The present study extends the previous work of Lailach, Thompson and Brindley (1968a, b) on the absorption of some purines, pyrimidines and their nucleosides by various cation-saturated montmorillonites. The

same general procedure has been employed, namely to measure by a uv-spectrophotometric method the depletion of the organic concentration of a solution due to the addition of a known amount of clay, with the pH of the equilibrating solution as the principal variable. To offset the smaller specific surface area and smaller cation exchange capacity of illite as compared with the corresponding data for montmorillonite, a high ratio of clay/organic has been necessary.

EXPERIMENTAL

Preparation of illite. An illite (Oswego illite) from Skytop, Pennsylvania, previously described by Weaver (1953), was selected since previous work (Wentworth, 1967) had shown this material to be well crystallized with few contaminants, excepting organic material. The gray color of the starting material suggested appreciable organic contamination and this was removed by repeated treatment with 30 percent H_2O_2 . Fe-hydroxy contaminants were removed by treatment with sodium citrate and sodium bicarbonate solutions. Both procedures followed the methods described by Jackson (1956). After washing the clay free of salts, it was fractionated to <1 micron. The resulting illite was nearly white in color and gave a considerably clearer x-ray powder diffraction diagram indicating a $2M_1$ -type crystal structure.

The purified illite was saturated with Na, Mg, and Cu(II) by washing each sample three times with the appropriate 1N salt solution, NaCl, $MgCl_2$, or $CuSO_4$. Na- and Mg-saturated illites were dialyzed at $60^\circ C$ with deionized water which was changed repeatedly until a negative Cl^- test was obtained. The Cu(II)-saturated illite was washed by centrifugation in order to minimize Cu-hydroxy formation; the washing was continued until a negative test for Cu(II) was obtained with NH_4OH , and two further washings were then given.

Cation exchange capacity. A 5 ml aliquot of a suspension of known concentration of each of the three clays was washed five times with 1N $CaCl_2$ solution and then washed with deionized water until a negative Cl^- test was obtained. The resulting Ca-saturated illites were washed five times with 1N NaCl in order to exchange the Ca, and the five washings were combined. The exchanged Ca was titrated against cyclohexane-diaminetetraacetic acid (CyDTA) with Calcein as the indicator. The cation exchange capacity of the three clays was determined to be 0.14 ± 0.01 meq/g; this corresponds to one Na^+ ion for every 77 \AA^2 of surface, or 0.61 Na^+ per unit-cell area, which can be compared with 1 K^+ ion per unit-cell area on an external face of muscovite.

Surface area. A gas absorption apparatus of the B.E.T. type was used for determining the surface area of the fractionated illite. The sample was outgassed at $80^\circ C$ and 10^{-6} mm Hg overnight. Krypton was used as the absorbate and the area was found to be $65 m^2/g$.

Organic absorption measurements. To 10 ml of 1.0 mM solutions of organic compounds and 5 ml of a clay suspension (40 g dry clay/l), various amounts of HCl, NaOH, and H_2O were added to give a final volume of 25 ml and equilibrium pH values ranging from about 2 to 10. Under these conditions, absorption rarely exceeded 90 percent and equilibrium was achieved in about ten hours. From the amounts of material used, it is easily calculated that complete absorption of the organic material corresponds to one molecule per 220 \AA^2 of the illite surface or one molecule per 5 unit cells. Evidently the available surface is sufficiently

large so that there can be no restriction on the absorption process by the amount of illite used.

After reaching equilibrium, the clay was centrifuged down using a Sorvail SS-1 high speed angle centrifuge. The pH values of the supernatant solutions were found with a Beckman Expandomatic pH meter. 5 ml aliquots of the supernatant solution were diluted to the mark in 100 ml volumetric flasks with pH 2 buffer. The non-absorbed organic material was determined using a Beckman DU spectrophotometer. The accuracy of measurement of the organic concentrations was better than 2 percent and the accuracy of the pH measurements was about 0.05 of a pH unit.

Organic materials used. For the most part, the same organic materials have been studied as were used in the previous work on absorption by montmorillonite (Lailach, *et al.*, 1968a,b).

RESULTS AND DISCUSSION

The results are summarized in Figures 1-5 where the percentages of organic material absorbed by the clay from the initial solutions are plotted against the pH of the equilibrating solutions, respectively for the various purines, pyrimidines and nucleosides studied. The curves show that the absorption depends on the pH of the solution, the exchangeable inorganic cations, Na, Mg and Cu, and the nature of the organic molecules.

The curves for Na- and Mg-illite generally are simpler than those for Cu-illite, and exhibit a single maximum in the acidic range of pH and mainly low absorption in the basic range. Hypoxanthine (6-hydroxypurine), see Figure 1, shows anomalous results as compared with those of the other purines examined; similar anomalies were obtained previously with montmorillonite, (see Figure 2). For Cu-illite, the curves are more varied in form and, in some cases, appreciable absorption occurs under basic conditions.

Absorption by Na- and Mg-illite; dependence on pK_a . The prominent absorption maxima exhibited by Na- and Mg-illite under acidic conditions point to cation exchange reactions between protonated organic molecules and illite as being the principal absorption mechanism. Several ion exchange reactions must be considered which can be represented as follows:



In these reactions, R stands for the organic molecule, RH for the protonated form, and X for a monovalent cation.

Of the four reactions listed, (1) is of primary importance because it

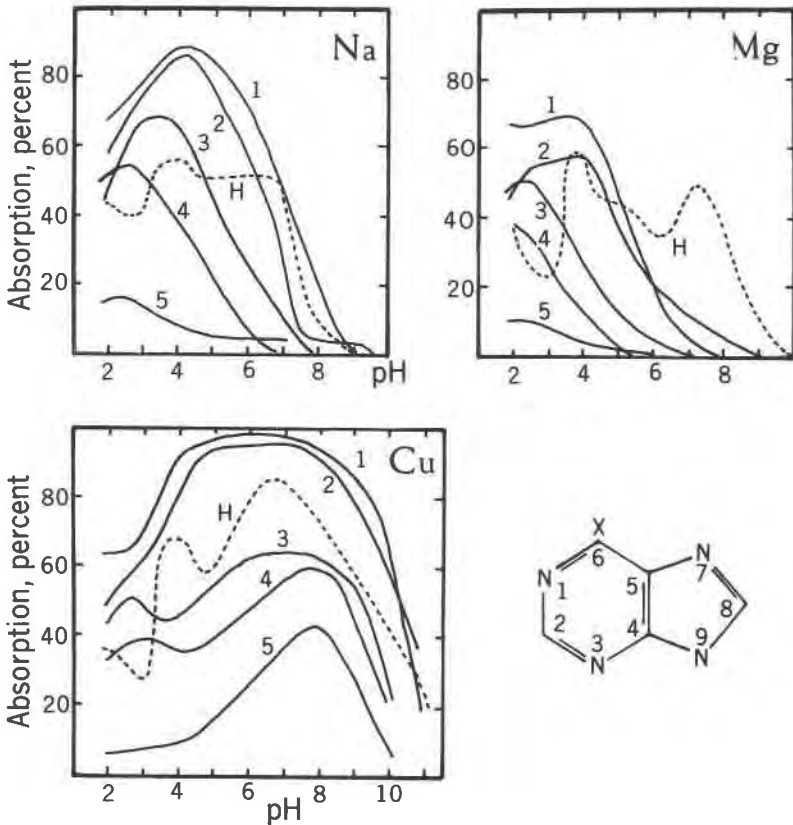


FIG. 1. Percentage absorption of purine and purine derivatives by Na-, Mg-, and Cu (II)-illite versus pH of equilibrating solutions. Organic compounds used are: (1) 6-amino 7-methylpurine, (2) 6-aminopurine (adenine), (3) 6-methylpurine, (4) purine, (5) 6-chloropurine, (H) 6-hydroxypurine (hypoxanthine).

furnishes the organic cations participating in reaction (2). Reaction (3) is important because under the acidic conditions where most of the absorption occurs, the illite may be largely in the hydrogen form. This has been checked for Na-illite reacting with a solution of adenine at pH 4. Analysis of the equilibrating solution showed that the Na^+ ions were fully displaced from the illite. If the absorbed adenine was wholly cationic, then under the conditions of the experiment, the exchange capacity of the clay was satisfied by 4.3 meq/g of adenine ions and the remainder, 9.7 meq/g, by hydrogen ions. The equilibrating reactions may be complicated further by some decomposition of the illite in acidic solutions with the liberation of other cations, such as Al^{3+} or hydroxy-Al ions.

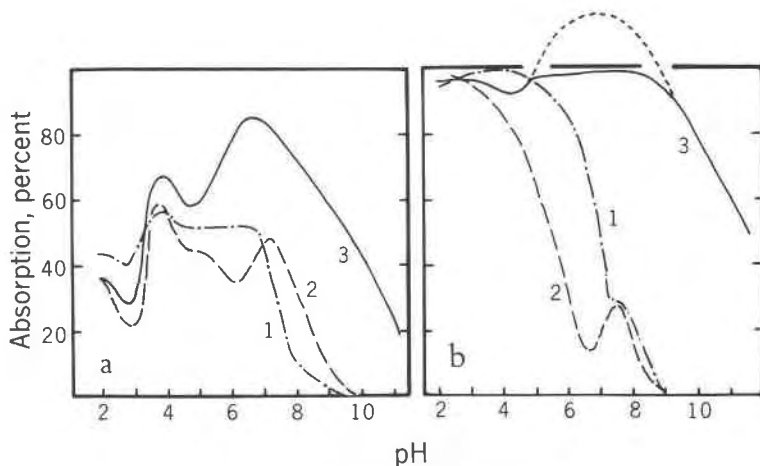


FIG. 2. Comparison of absorption curves for 6-hydroxypurine (hypoxanthine) by (a) Na-, Mg- and Cu(II)-illite, (b) Na-, Mg-, and Cu(II)-montmorillonite. In (b), the dashed line for Cu(II)-montmorillonite indicates the possible shape of the curve if absorption had not reached 100 percent in the pH range indicated. Curves 1, 2, 3 are respectively for Na, Mg and Cu(II) clays.

The extent to which organic cations are formed is governed primarily by the equilibrium constant K_a of reaction (1):

$$pK_a = \log ([RH^+]/[R]) + pH$$

Hence the ambient pH must be about equal to, or somewhat less than, pK_a to give considerable protonation of the organic molecules. When the pK_a value of the organic molecule is small, the ambient pH must be correspondingly small, but with increasing H ion concentration the illite will become increasingly H-illite by reactions (3) and (4), and organic absorption will decrease. It is easily understood, then, that a maximum absorption is likely to occur when $pH \sim pK_a$. For most of the molecules used, the pK_a values were given by Lailach *et al.* (1968a), but the following additional values have been determined: 6-methylpurine, 3.10; deoxyadenosine, 3.75; pyrimidine, 1.30; 2-aminopyrimidine, 3.54.

In Figure 6, the lower diagram shows the pH for maximum absorption taken from Figures 1, 3, 4 and 5 plotted against the pK_a values of the organic molecules, with open symbols for Na-illite and solid symbols for Mg-illite. For each type of illite, the pH for maximum absorption varies almost linearly with pK_a , with little or no dependence on molecular form. The absorption depends strongly on the inorganic exchangeable cation, Na or Mg, and the fact that the Mg-illite line falls below that of Na-illite reflects the greater difficulty of replacing Mg^{2+} than Na^+ cations.

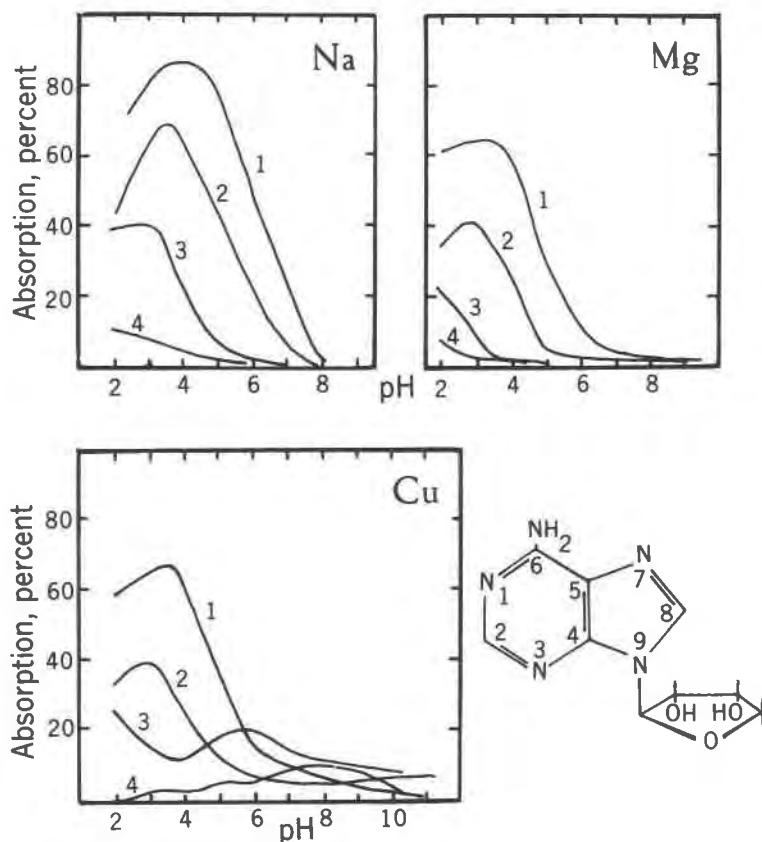


FIG. 3. Percentage absorption of (1) deoxyadenosine, (2) adenosine, (3) guanosine, (4) inosine, from aqueous solutions, by Na-, Mg-, and Cu(II)-illite, versus pH of equilibrating solution.

The upper diagram of Figure 6 indicates that the maximum absorption, A_{max} , depends on the organic molecular constitution as well as on pK_a . For purines and their nucleosides, the results fall on the upper straight line, while those for pyrimidines fall on the lower line. Evidently the larger size of the purine molecules leads to greater absorption probably because of van der Waals' interactions. In this diagram, data are shown for only Na-illite. For Mg-illite, the A_{max} values are fewer in number and are more scattered than those for Na-illite and when plotted on the same diagram confuse rather than clarify the picture.

Absorption by Cu(II)-illite; complex formation. The absorption curves for Cu(II)-illite are substantially different from those for Na- and Mg-illite

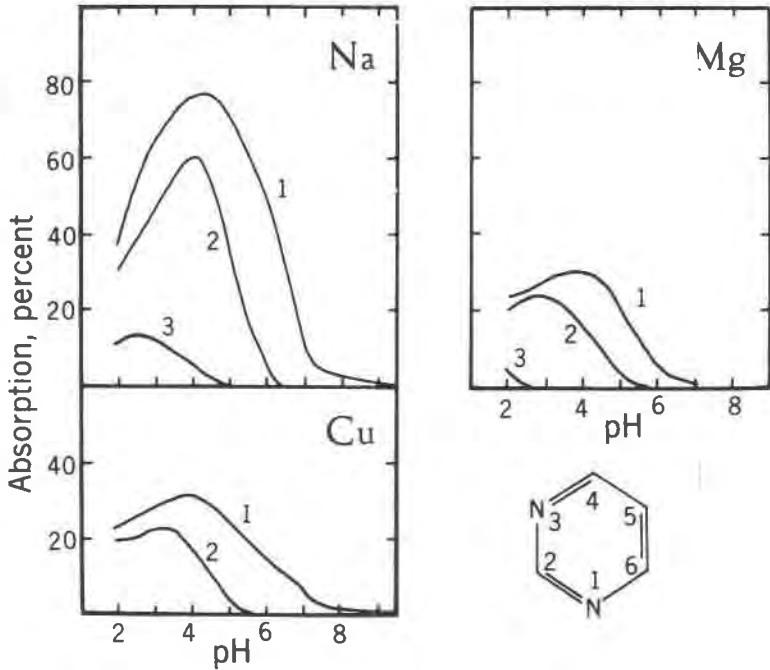


FIG. 4. Percentage absorption of (1) 4-amino 2-hydroxy pyrimidine (cytosine), (2) 2-aminopyrimidine, (3) pyrimidine, by Na-, Mg-, and Cu(II)-illite versus pH of equilibrating solutions.

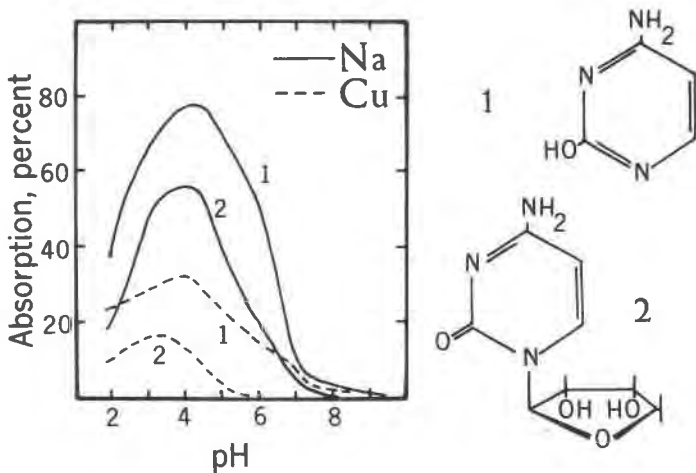


FIG. 5. Percentage absorption of (1) cytosine compared with that of (2) cytidine by Na- and Cu(II)-illite in relation to pH of equilibrating solution.

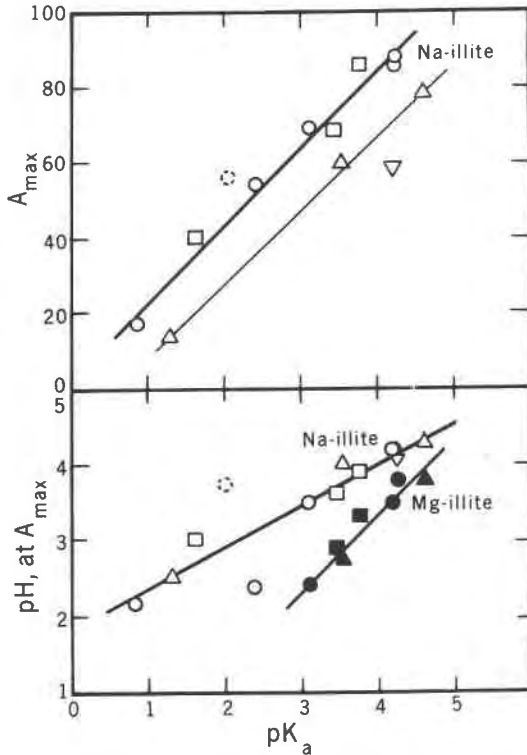


FIG. 6. Relations between pH giving maximum organic absorption, A_{max} , the maximum absorption, and pK_a values of organic compounds used. Open symbols give data for Na-illite, solid symbols for Mg-illite. Organic compounds are as follows: \circ purines, Δ pyrimidines, \square purine nucleosides, ∇ pyrimidine nucleoside.

and are analogous to those for Cu(II)-montmorillonite. Particularly for the purines (Figure 1), but also to a lesser extent for the nucleosides (Figure 3), absorption extends into the basic pH range. In some cases, *e.g.* 6-methylpurine and purine, double maxima are observed and in other cases, the maxima are so broad that possibly two absorption maxima overlap without resolution, *e.g.* 6-aminopurine. When two maxima occur, the one at lower pH is probably due to a cation exchange reaction, and the one at higher pH, around 6.5–8, observed mainly for the purines, probably indicates complex formation between the N-3 and N-9 positions of the purine rings and the Cu(II) ions, which has been discussed in some detail previously, (Lailach *et al.* 1968b).

The maximum absorptions of the purines by Cu(II)-illite in the pH range 6.5–8 can be correlated with the electron-releasing abilities of the

functional groups attached to the 6-position, (position X shown in Figure 1), with $\text{NH}_2 > \text{OH} > \text{CH}_3 > \text{H} > \text{Cl}$. This correlation can be treated quantitatively by using the coefficient σ_p which appears in the Hammett equation and which "is characteristic only of the substituent . . . and represents the ability of the group to attract or repel electrons . . ." (Gould, 1966; see pp. 220–221). A positive σ value for a substituent indicates that the substituent is a *stronger* electron attractor than hydrogen, while negative values of σ indicate electron releasing ability. The subscript 'p' relates to the para relation between X and N. For the groups in question here, the $-\sigma_p$ values are as follows: NH_2 , 0.66; OH , 0.46; CH_3 ,

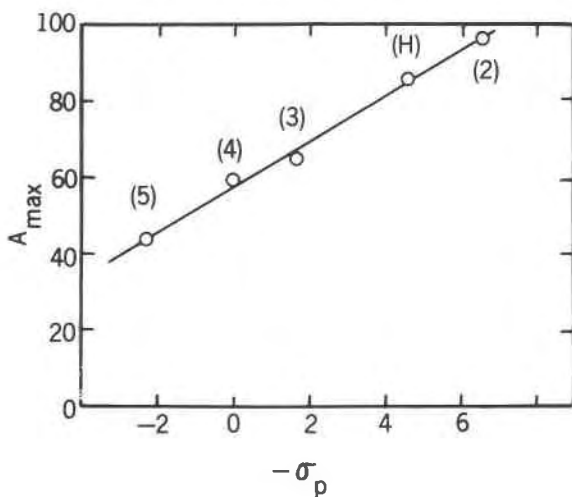


FIG. 7. Relation between maximum absorption of purines in the pH range 6.5–8 by Cu(II) -illite, and $-\sigma_p$, electron releasing coefficient of the substituent groups.

0.17; H, 0.00; Cl, -0.23. Figure 7 shows a linear relationship between maximum absorption in the pH range 0.56–8 and the coefficients $-\sigma_p$, and the designations of the points in the figure indicate the corresponding curves of Figure 1. The curve labelled '1' in Figure 1 is for 6-amino 7-methylpurine and this molecule is not strictly comparable with the others because of the additional group attached to the 7-position.

In the case of the pyrimidines, Figures 4 and 5, where the single aromatic ring excludes a similar complex formation, absorption by Cu(II) -illite closely resembles that by Mg - and Na -illite, i.e., it arises primarily by cation-exchange reaction. However, in the case of Cu(II) -montmorillonite (Lailach *et al.* 1968b,) it was considered that a *weak* complex with cytosine (2-hydroxy 4-aminopyrimidine) was probably formed and similar evidence is now obtained with Cu(II) -illite. In

Figure 4, it is seen that cytosine is absorbed by Cu(II)-illite further into the basic pH range than by Mg-illite, though in other respects the curves are similar. When the absorption of cytosine is compared with that of cytidine by Na- and Cu(II)-illite, it is evident that the blocking of the N1 position by the ribose ring greatly reduces the absorption over the whole pH range, but especially in the basic range.

Absorption of hypoxanthine. The absorption of this compound by illite and by montmorillonite is highly anomalous (see Figures 1 and 2). With a pK_a value of 1.98, hypoxanthine might be expected to behave in a manner similar to that of purine, with $pK_a = 2.39$. The high absorption of this compound by Cu(II)-montmorillonite possibly conceals an absorption maximum around pH7 which is sketched in hypothetical form in Figure 2b. In the previous work, hypoxanthine was the only compound showing a clear absorption maximum around pH = 7.5 with Mg- and Ca-montmorillonite and an unresolved but extended absorption by Na-montmorillonite. The same features now appear with illite. That this compound alone of those which have been studied shows marked absorption around pH7 suggests that the 6-hydroxy feature of the molecule plays some part in complex formation with the inorganic cations.

CONCLUSIONS

The mechanisms controlling the absorption of purines, pyrimidines and their nucleosides from aqueous solution on Na-, Mg-, and Cu(II)-illite are similar to those pertaining to their absorption by the corresponding montmorillonites. In the acidic pH range, the principal mechanism is a cation exchange reaction. The pH values for maximum absorption vary linearly with the pK_a values of the organic molecules and show little dependence on molecular constitution except in so far as the constitution determines pK_a . The maximum absorption also varies linearly with pK_a but also depends on molecular size, being larger for larger molecules probably because of van der Waals' interactions. Absorption by Cu(II)-illite takes place also at higher pH values, 6.5-8, probably by complex formation and appears to depend on the electron releasing ability of the functional groups attached to the organic molecules. A close linear correlation is shown between the maximum absorption and the coefficient $-\sigma_p$ appearing in the Hammett equation which is a measure of the electron-releasing ability of substituent groups.

The absorption characteristics shown by illite and discussed in this paper are more sharply delineated than those shown by montmorillonite, and it has been possible in the present work to consider the pH values at which maximum absorption occurs and to make correlations

with pK_a values. In the work on montmorillonite well-defined pH values for maximum absorption were, in many cases, not evident and of, necessity, those discussions were along more general lines.

ACKNOWLEDGMENTS

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