change orientation to accommodate a receptor. Interaction with tubulin might take place *via* hydrogen bonding with specificity occurring because the ether oxygen[15] can only hydrogen bond to an atom with hydrogen –NH or –OH.[16] Hydrogen bonding allows wide latitude in configuration. For example, O–H···O distances[17] may vary from about 2.5 to about 3.0 Å. Thus exact matching of distances in receptor and drug may not be necessary, and drugs where these distances vary may still be effective.

Other drugs which partially mimic colchicine are vinblastine and melatonin[11] which both fit criteria 1, 2, and 3 above. Vinblastine[18] contains a methoxy group 6.0 Å away from –OH which could correspond to either the 6.6- or 5.6-Å distance in colcemid. Melatonin[19] contains two –NH groups, one of them 5.5 Å from methoxy and the other found in crystals to be 7.1–7.9 Å from methoxy depending upon the conformation of the side chain. Inspection of models shows that the latter could change conformation and nicely match the 6.6-Å colcemid distance with good matching of the orientation of the –NH groups.

Acknowledgment. This work was supported in part by a grant from the National Science Foundation. I am indebted to Professor R. Rosenstein and Dr. H. Berman for their help with the Syd Hall method, to Professor L. Margulis for suggestions, and to the staff of the University of Massachusetts Research Computing Center.

Supplementary Material Available. A listing of structure factor amplitudes will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number JACS-74-899.

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The Dielectric Increments of Amino Acids

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Abstract: The dielectric increments for the first ten members of the homologous series of α,ω-amino acids, together with those for a number of rigid or "pseudorigid" molecules, have been calculated from dielectric constants in aqueous solution. The results suggest that the flexible molecules exist in their most extended conformations and that solvent effects contribute significantly to the observed dielectric increments.

The importance of the conformational behavior of hydrophobic chains in aqueous media has become apparent in a variety of fields.[1–6] In particular, the understanding of processes in biological and biomimetic systems requires clear descriptions of the preferred conformational states of these chains. Breslow has demonstrated very strikingly the criteria required in natural systems to transform, for example, stearic acid into oleic acid, introducing a double bond at a specific point in a homogeneous methylene chain.[7] Such specificity is clearly dependent on the predominant conformation(s) of the aliphatic chain. The ubiquity of amino acids in biological systems has resulted in a large body of work on their conformational behavior.[8,9] The physiological activity of these compounds is dependent upon the conformations they are able to assume.[10–13] In this paper we report the results (and interpretations thereof in terms of chain conformations) of a study of the dielectric constants of aqueous solutions of a larger series of α,ω-amino acids than has previously been examined.

Measurements of the dielectric constants of aqueous solutions of amino acids provided, in large measure, the evidence that such molecules exist in water as dipolar ions or zwitterions.[14] Although at the time at which these studies were carried out there was no exact theory to interpret the dielectric constants of polar liquids,

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empirical relationships proved to be extremely valuable. The earlier work showed that the dielectric constant, ϵ, of an aqueous solution of an amino acid is greater than that of water, and increases linearly with solute concentration, c, expressed as mol $l.^{-1}$.[15] Thus the results for any given molecule may be expressed as a "molar dielectric increment," δ, defined as the variation of ϵ with c, $\Delta\epsilon/\Delta c$. The value of δ is almost temperature and solvent invariant, and is constant over a range of frequencies (1–300 MHz), thus eliminating anomalous dispersion effects.[14]

The literature values of δ for amino acids have been summarized and discussed at length by Wyman,[14] Greenstein and Winitz,[8] and Cohn and Edsall.[9] The early results showed a linear variation of δ with n, the number of methylene carbon atoms in the chain of an α,ω-amino acid, for the first six members of the simple aliphatic series (eq 1).[14] More recent work on four

$$\delta = 13n + 11 \quad (1)$$

members of this series (n = 1, 2, 5, 8) also suggested a linear relationship between δ and n (eq 2).[16] These

$$\delta = 16.5n + 5 \quad (2)$$

results were interpreted by Kirkwood (ref 9, Chapter 12) as indicating that the polymethylene chains of the α,ω-amino acids were coiled rather than rigidly extended. He had modified the earlier theories of Debye and Onsager to allow the calculation of the dipole moments, μ, of polar molecules from their dielectric increments and derived an expression of the form[17]

$$\mu = 3.30\delta^{1/2} \quad (3)$$

This equation predicts a linear relationship between δ and the square of the length of the dipole in cases such as the amino acids where the magnitudes of the charges constituting the dipole are known. The root mean square end-to-end distance, R_{free} (and hence μ), was shown by Kuhn[18] and Eyring[19] to be proportional to $n^{1/2}$, if one assumed free rotation about each bond and no systematic relationship between the angles of rotation around successive bonds. In such a case, eq 3 requires a linear relationship between δ and n, in agreement with the empirical findings reported to date and summarized in eq 1 and 2, whereas it would require a linear relationship between δ and n^2 if the hydrocarbon chains were fully extended. These results have been widely quoted as indicating that α,ω-amino acids behave in aqueous solution as freely rotating chains, their mean end-to-end distances being less than those for full chain extension. In this paper, we show from an examination of the dielectric increments of a larger series of α,ω-amino acids that this conclusion is erroneous.

Experimental Section

Materials. **α,ω-Amino acids** were commercially obtained, or prepared in accordance with literature methods, and recrystallized at least three times from water. Their melting or decomposition points agreed with the literature values.

4-Aminobicyclo[2.2.2]octane-1-carboxylic acid was made by combining the methods of Kauer,[20] Chapman, *et al.*,[21] and Roberts *et al.*,[22] and, after recrystallization from water, had mp >360° (lit.[22] mp 365°).

***cis*- and *trans*-2-Aminocyclopentane-1-carboxylic Acid.** A 50% mixture of the methyl and ethyl esters of 2-cyclopentanonecarboxylic acid (Aldrich) was converted into the corresponding mixture of oximes by the standard method for water-insoluble ketones.[23] After distillation the mixture (25 g) was hydrogenated at 900 psi over freshly prepared Raney nickel (3 g) in absolute ethanol (200 ml) for 2.5 hr. The crude product, after filtration and removal of solvent *in vacuo*, was refluxed with distilled water (350 ml) for 24 hr and the solvent removed. The residual mixture of isomers was separated by fractional crystallization from water, the trans isomer being less soluble than the cis isomer. Recrystallization from water initially yielded *trans*-2-aminocyclopentane-1-carboxylic acid, mp 253–255° (lit.[24] mp 240°). Evaporation of part of the solvent gave *cis*-2-aminocyclopentane-1-carboxylic acid, mp 205–206° (lit.[24] mp 202–204°).

Water. Doubly distilled water was passed through a mixed-bed ion-exchange resin (Rexyn I300) immediately before use. The specific conductivity was approximately 10^{-6} ohm^{-1} cm^{-1} at 25°.

Calibration Solvents. Acetone and nitrobenzene were treated as described previously.[25]

Results

Measurements. Dielectric constants of aqueous solutions were measured using a Dipolemeter, Type DM01 (Wissenschaftlich-Technische Werkstätten, 2 MHz), fitted with a water-jacketed cell, Type MFL3/S, and observing the precautions concerning conductivity and purity described in an earlier paper.[25] All measurements were made at 25.00 ± 0.01° except for 11-aminoundecanoic acid which, because of its low solubility, was studied at 40.00 ± 0.01°. (The low temperature variation of δ for glycine was assumed to be typical of this series of compounds.) Constant temperatures were maintained by circulating water from an external bath. Solutions were made up by weight and molarities calculated from molalities and solution densities.[25] Care was taken to ensure thermal equilibration and readings were taken over a 15-min period, the values being averaged to calculate the dielectric constant.

Data Treatment. The dielectric constant of each amino acid solution was calculated from the capacitance readings and the slope and intercept of the calibration plot. From the linear variation of dielectric constant with molar concentration the dielectric increment was obtained. Values of the dielectric increments for the compounds studied are given in Tables I and II and the variation with chain length for the flexible molecules, as represented by n, is shown in Figure 1.

The values of R, the intercharge distance in rigid molecules, and of R_{max}, the intercharge distance in flexible chains in their most extended conformation, were calculated from known bond lengths and angles, with the aid of Courtauld models. The charge on the carboxyl group was assumed to lie symmetrically between the oxygen atoms on the extension of the methylene–carboxyl bond. The charge on the ammonium

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Table I. Dielectric Increments, δ, and Extended-Chain Lengths, R_{max}, for Some Flexible Amino Acids in Water at 25°

Amino acid	R_{max}, A	δ	Correlation[a] coeff	Lit. values[8,9,14]
Glycine	3.25	24.0 ± 0.6	0.995	22.6, 26, 23.7
β-Alanine	5.07	27.9 ± 0.2	0.986	35, 33, 33.3
4-Aminobutyric	5.76	38.9 ± 0.5	0.999	51, 55
5-Aminovaleric	7.58	58.9 ± 0.1	0.997	63
6-Aminohexanoic	8.27	74.6 ± 0.2	1.000	78, 73, 82.3
7-Aminoheptanoic	10.09	93.6 ± 0.2	0.999	87
8-Aminooctanoic	10.79	109.4 ± 0.1	0.999	
9-Aminononanoic	12.61	130.8 ± 0.5	1.000	144.7
10-Aminodecanoic	13.31	152.9 ± 0.3	0.998	
11-Aminoundecanoic[b]	15.12	195 ± 2	0.93	

[a] For the variation of dielectric constant with concentration. [b] Studied at 40°.

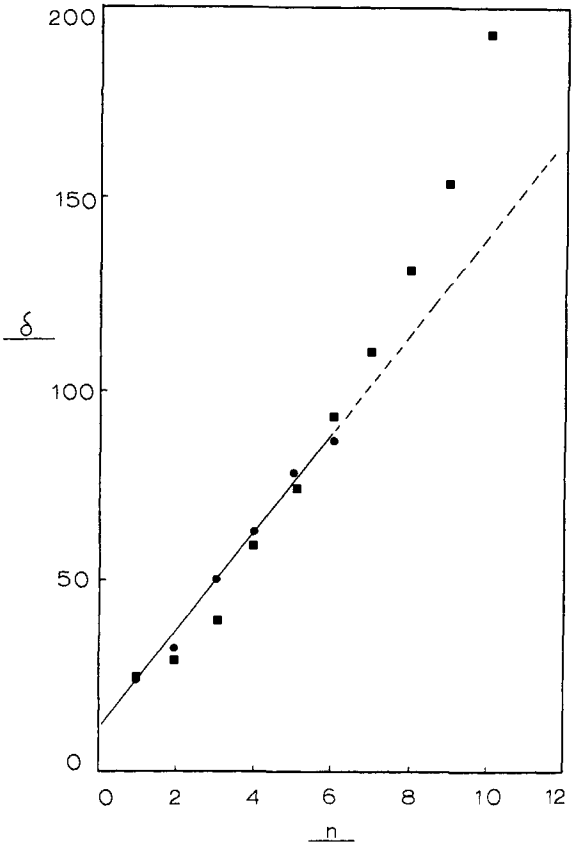


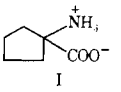
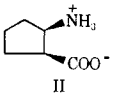
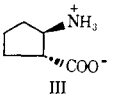
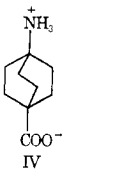
Figure 1. The variation of the dielectric increment, δ, with the number of carbon atoms between the charged groups, n. The linear relationship plotted is eq 1: (■) data from Table I; (●) data from ref 1.

group was taken to lie 0.25 Å beyond the nitrogen atom on the extension of the carbon–nitrogen bond. The intercharge distances, R, in the 1,2-cyclopentane derivatives were calculated for the conformations with greatest charge separation. Values of R and R_{max} are included in Tables I and II and the linear relationship between δ and R_{max}^2 is shown in Figure 2.

Discussion

From Figure 1 it can be seen that the variation of δ with n becomes increasingly nonlinear with increasing chain length. Our results for the first five members of the series lie very close to earlier results, with the exception of the results for 4-aminobutyric acid; we are unable to provide an explanation for this discrepancy.

Table II. Dielectric Increments, δ, and Intercharge Distances, R, for Some Rigid Amino Acids in Water at 25°

Amino acid	R, Å	δ	Correlation[a] coeff
 I	3.2	19.6 ± 0.5	0.996
 II	3.0	16.15 ± 0.02	0.977
 III	4.8	23.2 ± 0.09	0.981
 IV	7.1	71.6 ± 0.05	0.999

[a] See Table I, footnote *a*.

When $n > 6$, increasingly positive deviations from eq 1 are observed (Figure 1). Similar behavior was found by Gaumann and Gunthard,[16] although their results do not agree with ours, perhaps because their purification technique may have led to some dehydration and polyamide formation. However, it is clear that the linear relationship between δ and n found by these earlier workers arose from examining only the shorter α,ω-amino acids, and that the previous conclusions regarding the conformations of these molecules in solution must be rejected.

Equation 3 indicates that δ is proportional to the square of the dipole length of an amino acid and we have accordingly plotted the values of δ against R_{max}^2 in Figure 2, where R_{max} is the intercharge distance assuming maximum chain extension. From this plot it is evident that an excellent correlation exists (correlation coefficient = 0.997) and we consider this to imply that the α,ω-amino acids are in their fully extended forms in aqueous solution. These results might appear surprising, in view of the evidence that, in inert solvents, straight-chain molecules exist in a variety of conformations, the rigidly extended conformation forming a decreasing proportion of the whole as n

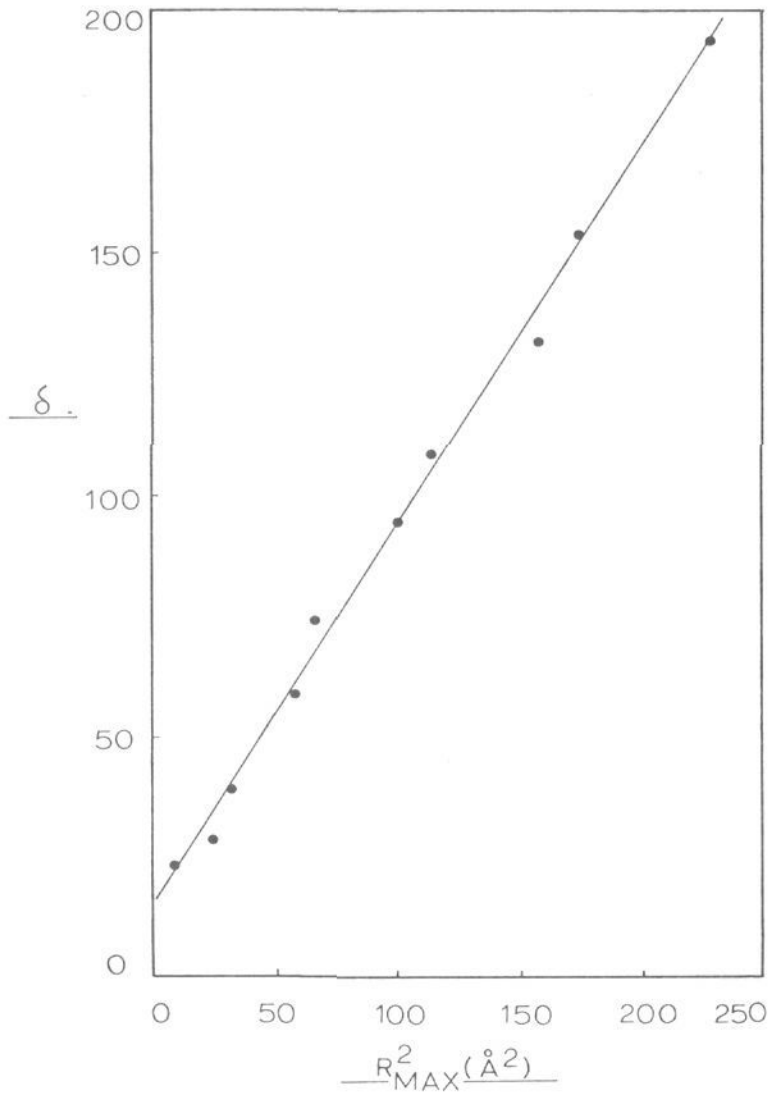


Figure 2. The variation of the dielectric increment, δ, with the square of the extended-chain length, R_{max}^2.

increases.[26–29] Although this is an acceptable notion for straight-chain molecules in the gas phase, or in comparatively inert solvents, it is unlikely to be correct for zwitterions in polar solvents such as water. In this case (as has already been pointed out[30]) the very strong interaction between the charged ends of zwitterions with the dipoles of the solvent water molecules will cause the latter to cluster about the ends of the molecules and to extrude as completely as possible the less strongly attracted hydrocarbon chain. This results in the chain assuming its most extended conformation.[31]

The orientation of the solvent dipoles about the zwitterion results in a solvent contribution to the observed dielectric increment (Figure 3). Its importance is apparent from a comparison of the dielectric increments reported in Tables I and II. The distance R separating the charges of 4-aminobicyclo[2.2.2]octane-1-carboxylic acid (IV of Table II) would lead one to expect a dielectric increment of 55 from Figure 2; in fact, it is 30% greater. This can be understood by considering the effect of the solvent dipoles shown in Figure 3: those at the ends A and C of the zwitterion are oriented in such a way as to enhance the dipole

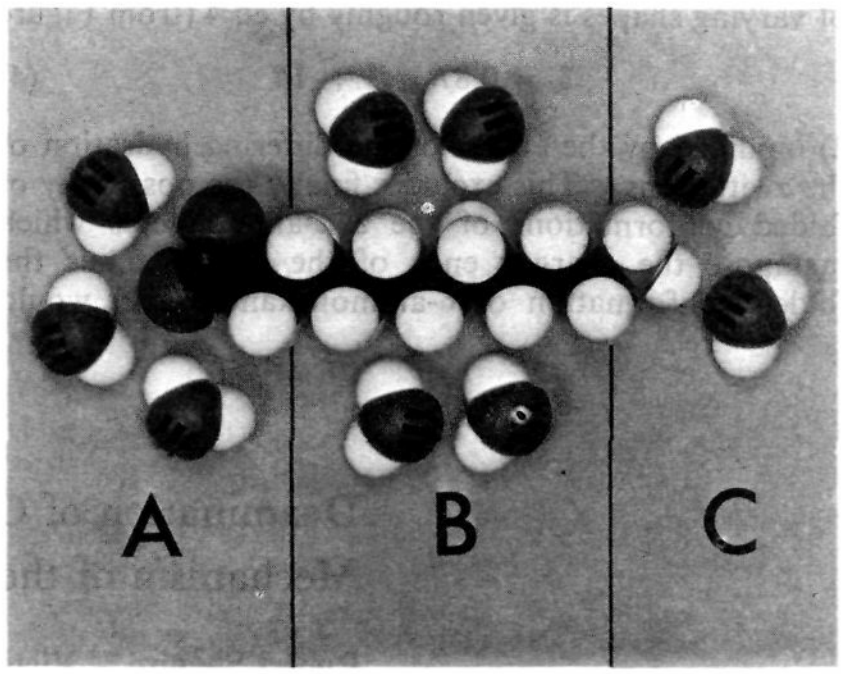


Figure 3. A schematic representation of the solvent orientation around 10-aminodecanoic acid (zwitterion form).

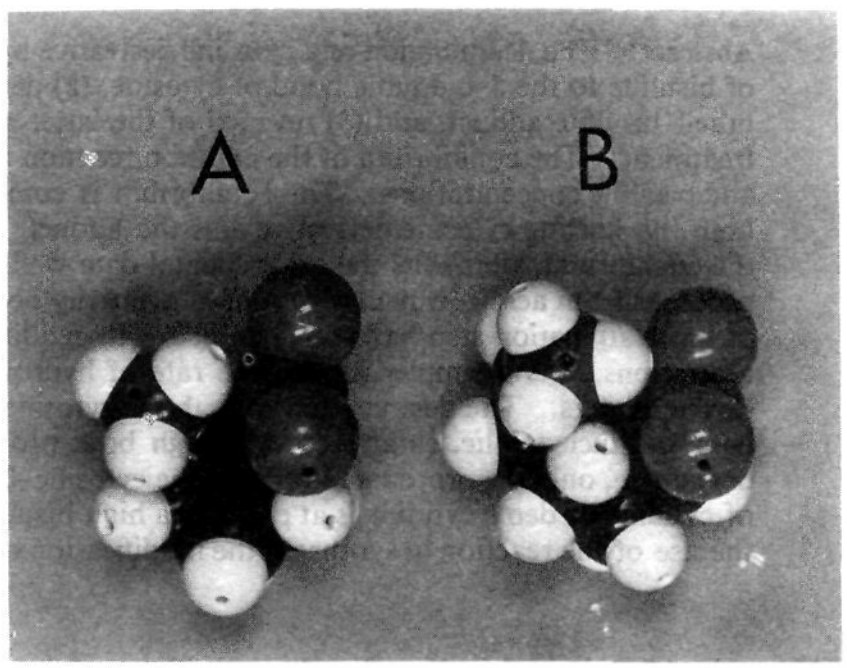


Figure 4. A comparison of *cis*-2-aminocyclopentane-1-carboxylic acid (A) with the folded conformation of 6-aminohexanoic acid (B).

moment of the zwitterion; those in the mid-region B in such a way as to oppose the dipole moment of the zwitterion. The chief difference between IV and the straight-chain α,ω-amino acids is that the former has a much thicker hydrocarbon structure in the region B, and hence excludes water molecules which would reduce the apparent dipole moment, and hence the dielectric increment.

A different effect is shown by 1-aminocyclopentane-1-carboxylic acid (I of Table II), which has a dielectric increment 18% lower than that of glycine (Table I). In both of these zwitterions the charged ends are so close together that the mid-region B of Figure 3 has vanished; consequently, the effect of replacing the hydrogen atoms of glycine by the cyclotetramethylene chain of A is to exclude some solvent from regions A and C, and hence to diminish the dielectric increment. The same considerations apply to amino acid II, which has the charged ends even closer together, and which has δ about 25% less than expected from Figure 2; on the other hand, III lies between I and IV, and has roughly the δ expected.

In summary, the dielectric increment for zwitterions

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of varying shapes is given roughly by eq 4 (from Figure 2) modified by the effects of the differing hydration of the zwitterions. This excludes finally the possibility of folded conformations of the α,ω-amino acids which juxtapose the charged ends of the chain. Thus the folded conformation of 6-aminohexanoic acid would have a shape very similar to that of II (Figure 4) and hence a dielectric increment of about 16, instead of 74.6 as observed. The data also show that substitution of the α-carbon atom by bridging methylene groups markedly affects the δ value, in contrast to the effects of alkyl substitution reported by Wyman.[14]

$$\delta = 0.8R_{max}^2 + 14 \quad (4)$$

Acknowledgment. We are grateful to the National Research Council of Canada for financial support.

Deamination of Cytosine Derivatives by Bisulfite. Mechanism of the Reaction

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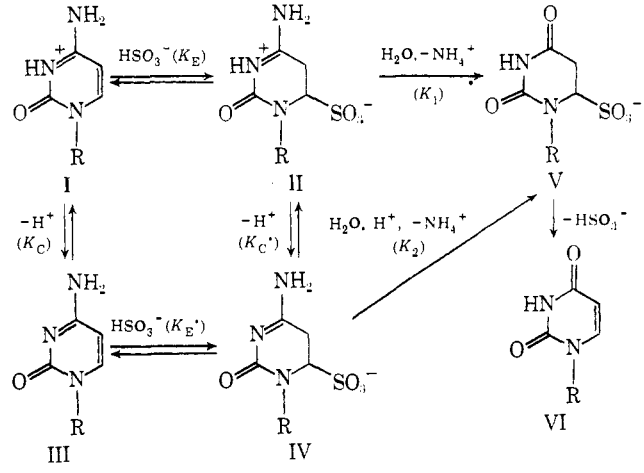
Contribution from the Department of Chemistry, New York University, New York, New York 10003. *Received September 4, 1973*

Abstract: The deamination of a cytosine derivative by sodium bisulfite involves the following steps: (1) addition of bisulfite to the 5–6 double bond of cytosine, (2) deamination of the resulting cytosine–bisulfite adduct to give a uracil–bisulfite adduct, and (3) reversal of the latter adduct to give a uracil derivative, by a subsequent alkaline treatment. The equilibrium of the addition reaction for cytidine has been determined at a number of pH values and bisulfite concentrations. The equilibrium is complex, as it involves protonated and unprotonated forms of both the starting cytosine derivative and the adduct. A mathematical expression has been derived to describe the equilibrium, which fits the experimental data well. The amount of adduct present at equilibrium falls as one proceeds from acidic to neutral solution, primarily because of the dissociation of bisulfite to sulfite. The kinetics of the deamination step have been studied with cytidine and deoxycytidine at various pH values and bisulfite concentrations. As a simpler model, the rate of hydrolysis of 1-methyl-5,6-dihydrocytosine has been examined in several buffers. The deamination of both the cytosine nucleosides and the model compound is subject to general base, or nucleophilic, catalysis. Although both protonated and neutral forms of 1-methyl-5,6-dihydrocytosine deaminate, only the protonated form of the cytosine–bisulfite adduct is reactive. The optimal rate of deamination of cytidine or deoxycytidine is at pH 5, in a high bisulfite concentration. These conditions are recommended for the use of the reaction in synthesis, the modification of nucleic acids, and chemical mutagenesis.

The use of sodium bisulfite for the specific deamination of cytosine derivatives was first reported in 1970 by ourselves[2] and by Hayatsu and coworkers.[3] In the intervening few years, the procedure has been widely applied for the chemical modification of nucleic acids and their components.[4] Our initial prediction of the mutagenicity of sodium bisulfite has been confirmed in four microorganisms.[5] These findings have raised the possibility that bisulfite may be an environmental mutagen, as it is used as an additive to foods, beverages, and pharmaceuticals[6] and is also the aqueous form of the common atmospheric pollutant, sulfur dioxide.

An understanding of the mechanism of this reaction is obviously important in determining the best conditions for the synthetic and genetic use of bisulfite, and in evaluating the possible hazard it presents as an environmental mutagen. The separate steps involved (Scheme I) have been given in earlier papers,[2,3] and

Scheme I



represent a specific example of the hydrolytic deamina-

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