A Solvent-Sensitive Aromatic Cotton Effect in Lysozyme[1]

Sir:

Evidence has accumulated in recent years that certain enzyme–substrate interactions may involve a deformation of portions of the enzyme at the active site.[2] If such a deformation were to involve an aromatic amino acid residue it might be susceptible to investigation by optical rotatory dispersion (O.R.D.), since conformation-dependent Cotton effects involving such chromophoric residues have been reported.[3] For example, in tobacco mosaic virus protein a small anomalous dispersion near 280 mμ could be resolved from the background rotation arising from the α-helix content.[4] Further, Myers and Edsall[5] have found Cotton effects in the region of the aromatic absorption bands in the carbonic anhydrases.

In this communication we report the presence of a Cotton effect centered near 280 mμ in egg-white lysozyme. This Cotton effect disappears on exposure of the enzyme to sodium dodecyl sulfate (SDS) at concentrations which *do not* affect the *α-helix content* of the molecule but which completely inhibit the enzymic activity. On the other hand, exposure of the enzyme to concentrated urea in acid solution results in the elimination of this Cotton effect as well as in the disruption of the α-helical portion of the molecule. Concentrated aqueous ethylene glycol solutions enhance the aromatic Cotton effect, with little effect on the rotation ascribable to the α-helix content.

O.R.D. measurements were made with a modified Bendix Polarmatic recording spectropolarimeter. Spurious effects due to the rotational response characteristics of the instrument in regions of rapidly changing optical density were checked by varying sample concentrations and cell path lengths. Over the wave length regions reported here the rotations were proportional to concentration and are therefore considered to be qualitatively genuine. Representative data are displayed in Figure 1, where optical rotation is plotted against wave length. To convert the relative rotation to a conventional [R′] at any wave length, the number must be multiplied by the Verdet correction $(28 \times 10^4)/(\lambda^2 - 1.72 \times 10^4)$ as well as the usual path length, concentration, and refractive index parameters.[6] This has been done for the four values at 233 mμ.

From the position of the positive Cotton effect in the 280-mμ region of lysozyme it can be inferred that this effect originates from the tryptophan residues, although a contribution from tyrosine residues cannot be excluded. The enhancement of the rotations in aqueous ethylene glycol solutions would suggest that

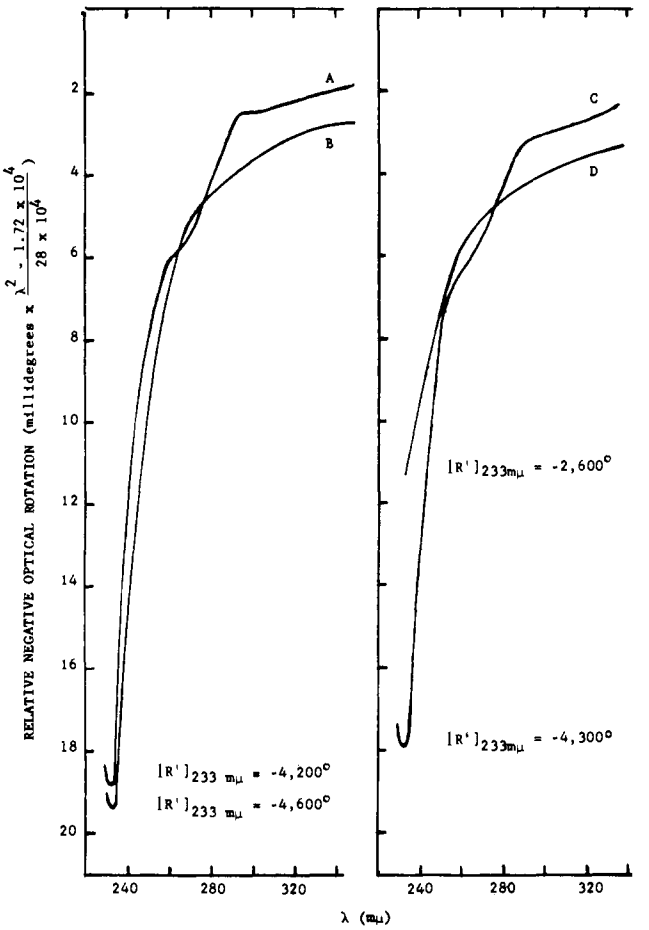

Figure 1. The ultraviolet optical rotatory dispersion of 0.178% lysozyme (Calbiochem Lot No. 3899) in a 0.16-cm. cell in: A, 76% ethylene glycol; B, 2.8% sodium dodecyl sulfate (recrystallized from isopropyl alcohol); C, 0.01 *M* KCl; and D, 7.2 *M* urea at pH 2.5.

the residues from which it originates are accessible to solvent perturbations. Four of the six tryptophan residues in native lysozyme have been found by the solvent perturbation technique to be accessible to a variety of solvents including ethylene glycol.[7,8] The magnitude of this observed effect may be due to a small conformational change involving exposed tryptophan residues, to a solvent (interaction) effect,[9] or, most likely, to a combination of both.

As may be seen in curve B exposure of the enzyme to SDS leads to the elimination of the 280-mμ Cotton effect with virtually *no effect* on the depth of the 233-mμ trough. Thus, interactions of the tryptophan residues responsible for the 280-mμ Cotton effect are independent of the integrity of the α-helical portions of the molecule (which from these data we estimate at ~25%).[10]

Since SDS has been shown to be a powerful inhibitor of lysozyme activity[11] and our data indicate a profound effect on the conformation of certain trypto-

(1) This work was supported in part by Grant No. GM 11061, Career Award No. 1-K6-DE-1094-01 from the National Institutes of Health, U. S. Public Health Service, and Contract AT(04-1)GEN-12 between the U. S. Atomic Energy Commission and the University of California.

(2) D. E. Koshland, Jr., *Cold Spring Harbor Symp. Quant. Biol.*, **28**, 473 (1963).

(3) See, for example, J. A. Schellman and C. Schellman, "The Proteins," Vol. II, H. Neurath, Ed., Academic Press Inc., 1964, p. 84.

(4) N. S. Simmons and E. R. Blout, *Biophys. J.*, **1**, 55 (1960).

(5) D. V. Myers and J. T. Edsall, *Proc. Natl. Acad. Sci. U. S.*, **53**, 169 (1965).

(6) See, for example, G. D. Fasman, *Methods Enzymol.*, **6**, 928 (1963).

(7) K. Hamaguchi and A. Kurono, *J. Biochem.* (Tokyo), **54**, 111, 259 (1963).

(8) K. Hayashi, T. Imoto, and M. Funatsu, *ibid.*, **55**, 516 (1964).

(9) K. M. Wellman, P. H. A. Laur, W. S. Briggs, A. Moscowitz, and C. Djerassi, *J. Am. Chem. Soc.*, **87**, 66 (1965).

(10) N. S. Simmons, C. Cohen, A. G. Szent-Gyorgyi, D. B. Wetlaufer, and E. R. Blout, *ibid.*, **83**, 4766 (1961).

(11) C. McLeod, *Am. J. Hyg.*, **34**, 51 (1941).

phan residues, it is compelling to consider these two phenomena may be related. In this connection Hayashi, *et al.*,[12] have shown that a tryptophan residue is involved in the formation of the enzyme–substrate complex between lysozyme and poly-N-acetylglucosamine (glycol chitin). Further, it has been reported by Hartdegen and Rupley[13] that an inactive derivative, altered only in a single tryptophan residue, may be obtained by oxidation of lysozyme by iodine in acidic solution. This modification and loss of activity were prevented by the presence of N-acetylglucosamine.

Further improvement in instrumentation will be necessary before more accurate measurements of the small aromatic Cotton effects can be obtained. Efforts in this direction are now being made and further results will be published in due course.

(12) K. Hayashi, T. Imoto, and M. Funatsu, *J. Biochem.* (Tokyo), **54**, 381 (1963).
(13) F. J. Hartdegen and J. A. Rupley, *Biochim. Biophys. Acta*, **92**, 625 (1964).

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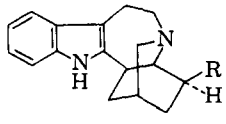
The Synthesis of Desethylibogamine

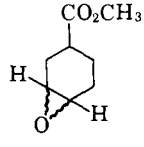
Sir:

We wish to report the synthesis of desethylibogamine (Ia), the first totally synthetic compound containing the carbon skeleton of the iboga alkaloids of which ibogamine (Ib) is a typical example.[1]

Oxidation of methyl 3-cyclohexene-1-carboxylate with *m*-chloroperbenzoic acid gives a mixture of the *trans* and *cis* epoxides (IIa and IIb,[2] b.p. 60–62° (0.1 mm.)).[3] Reaction of this mixture of epoxy esters with tryptamine in refluxing ethanol gave a mixture of amino alcohols[4] which was, without separation, heated at 190 to 200° to effect cyclization of III to the N-indolylethylisoquinuclidone (IV). Under these conditions, the amino alcohol from the *cis* epoxide would give either a

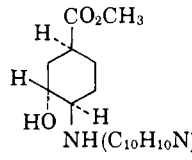


Ia, R = H
b, R = C_2H_5

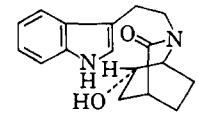


IIa, *trans* epoxide
b, *cis* epoxide

(1) J. P. Kutney, R. T. Brown, and E. Piers, *J. Am. Chem. Soc.*, **86**, 2287 (1964), have obtained a compound containing the ibogamine ring system from the mercuric acetate oxidation of carbomethoxydihydrocleavamine.
(2) Vapor phase chromatography of this mixture indicates that there is a predominance of one isomer (probably IIa), but the degree of separation was insufficient to permit a quantitative evaluation of the relative amounts of each isomer. H. B. Henbest and B. Nicholls, *J. Chem. Soc.*, 221 (1959), report that the oxidation of methyl 3-cyclohexene-1-carboxylate with perbenzoic acid gives exclusively the *trans* epoxide (IIa).
(3) Satisfactory analytical data were obtained for all new compounds reported, and all compounds were characterized by infrared and nuclear magnetic resonance spectroscopy.
(4) The infrared spectrum of this mixture indicates that a negligible amount of conversion to the amide was obtained.



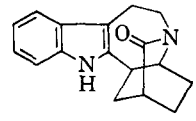
III



IV

δ-lactone and/or polymeric amide, and to separate the isoquinuclidone from these undesired compounds the crude mixture was heated with 5% methanolic sodium hydroxide[5] to give IV (m.p. 178–179°, λ_{max}^{KBr} 6.05 μ) in an over-all yield of 68%. The tosylate of this isoquinuclidone (m.p. 149–150°, 84% yield)[6] on treatment with aluminum chloride or aluminum bromide in toluene affords desethylibogamine lactam (V, m.p. 313–315°, λ_{max}^{KBr} 6.10 μ) in 38% yield. This compound gives a negative Ehrlich test, has a typical indole ultraviolet spectrum (λ_{max} 225, 283, and 291 mμ), and the n.m.r. spectrum is similar to that of ibogaine lactam.[5] The attempted use of a variety of other acids to effect this cyclization led to either gross decomposition or gave recovered starting material.

Reduction of desethylibogamine lactam with lithium aluminum hydride gives desethylibogamine (I, m.p. 186–187°, λ_{max} 226, 283, and 290 mμ) in 85% yield. The n.m.r. spectrum of this compound shows a series of peaks equal to approximately eight protons in the region from τ 6.6 to 7.2. Ibogamine and ibogaine both show a series of peaks in this general region which may be assigned to the protons adjacent to the indole and the tertiary nitrogen.



In addition to providing a synthetic pathway to the iboga alkaloid ring system in relatively few steps, this work constitutes a new isoquinuclidine synthesis which has been used to prepare the unsubstituted and N-benzyl analogs of V.[7]

Acknowledgments. This work was supported by Grant NB-04589 of the National Institute of Neurological Diseases and Blindness. We wish to thank Dr. Neville Finch of Ciba Pharmaceutical Company for samples of ibogamine and N-benzylisoquinuclidone which were used as spectral references.

(5) M. F. Bartlett, D. F. Dickel, and W. I. Taylor, *J. Am. Chem. Soc.*, **80**, 126 (1958), have pointed out the resistance of ibogaine lactam to basic hydrolysis.
(6) A second compound, $C_{31}H_{32}N_2S_2O_6$, m.p. 245–246°, is obtained if a large excess of tosyl chloride is used. The nature of this compound will be discussed in the full paper.
(7) J. W. Huffman, C. B. S. Rao, and T. Kamiya, unpublished work.

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Chemical Evidence for the Occurrence and Temperature Independence of Ion–Molecule Reactions at Atmospheric Pressures

Sir:

Abundant physical evidence for the occurrence of ion–molecule reactions in mass spectrometers has been