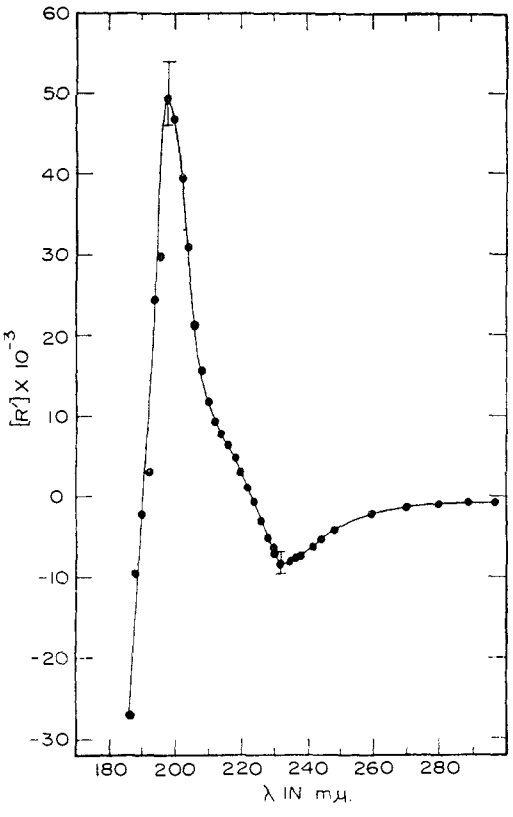

Fig. 2.—The ultraviolet optical rotatory dispersion of native bovine serum albumin in water solution, pH 7.1. A 1-mm. cell was used. Concentrations ranged from 0.0083 to 0.892%. Instrument use was as in Fig. 1.

The optical rotatory dispersion data for bovine serum albumin (Fig. 2), like helical polyglutamic acid, show two Cotton effects. This protein has been estimated to have between 38 and 58% helix by other methods[8]; the observed magnitudes of both the 225 mμ and the 190 mμ Cotton effects indicate a helix content of 55 to 60%. We have also confirmed the presence of the 190 mμ Cotton effect by measurements of other water-soluble synthetic polypeptides capable of undergoing helix → random transformations[9] and of other proteins.

The new 190 mμ Cotton effect is quite evidently related to the strong π–$\pi^*(NV_1)$ transition of amides and polypeptides whose absorption maximum lies around 190 mμ.[10,11,12] It has been known for some time that the rotatory strength of optically active absorption bands is not entirely determined by the intensity of absorption.[13] In this connection it is interesting that in the work presented here the ratio of the magnitude of the rotation to the extinction coefficient is larger for the 225 mμ Cotton effect than for the 190 mμ Cotton effect, although the size of the rotations observed with the 190 mμ Cotton effect is severalfold greater. Finally it should be noted that as yet it has not been possible to obtain reliable measurements below 184 mμ but extrapolation of our data indicate that the trough of the 190 mμ Cotton effect should be between 175 and 182 mμ. Further details and results with other materials will be published in due course.

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(8) For original references see a recent review by P. Urnes and P. Doty in "Advances in Protein Chemistry," Vol. 16, Edited by C. B. Anfinsen, N. L. Anson, K. Bailey and J. T. Edsall, Academic Press, New York, N. Y., 1961, p. 401.

(9) R. K. Kulkarni and E. R. Blout, to be published.

(10) W. T. Simpson and D. L. Peterson, *J. Chem. Phys.*, **26**, 588 (1957).

(11) K. Imahori and J. Tanaka, *J. Mol. Biol.*, **1**, 359 (1959).

(12) K. Rosenheck and P. Doty, *Proc. Natl. Acad. Sci., U. S.*, **47**, 1775 (1961).

(13) See for example W. Kuhn, *Ann. Rev. Phys. Chem.*, **9**, 417 (1958).

THE OPTICAL ACTIVITY OF POLYPEPTIDES IN THE FAR ULTRAVIOLET

Sir:

The optical rotatory dispersion of polypeptides and proteins, which has been usefully related to their conformation,[1] must have its origin in ultraviolet electronic transitions. A complete explanation of the dispersion in relation to conformation requires knowledge of both spectra and optical activity in the far ultraviolet. For the α-helical conformation, spectra[2] reveal two bands (at 191 and 206 mμ) due to exciton splitting of the $\pi \rightarrow \pi^*$ band and apparently a third, at 222 mμ, probably an $n \rightarrow \pi^*$ transition. Rotatory dispersion has, however, been measured to only 212 mμ. This reveals complex dispersion in the visible and, moreover, shows a negative Cotton effect with a trough at 233 and a crossover at 225 mμ.[3,4] Whereas Moffitt's analysis[5] assumes that the complex dispersion arises solely from $\pi \rightarrow \pi^*$ transitions, the location of this Cotton effect has led to the suggestion[2,3] that the $n \rightarrow \pi^*$ transition might be predominantly involved.

For the case of the disordered polypeptide chain, the spectra reveal only the 192 mμ transition and the rotatory dispersion remains simple above 216 mμ.

In order to assess optical activity at shorter wave lengths, we have measured circular dichroism by an adaptation of a Beckman DK-2A spectrophotometer. Circular dichroism possesses the advantage of intrinsic discreteness and can be transformed to rotatory dispersion. Circularly polarized light was produced in the spectrophotometer sample beam by a Rochon prism and quarter-wave plate, both of cultured quartz.[6] Measure-

(1) P. Urnes and P. Doty, *Adv. Prot. Chem.*, **16**, 401 (1961).

(2) W. B. Gratzer, G. Holzwarth and P. Doty, *Proc. Natl. Acad. Sci. U. S.*, **47**, 1785 (1961).

(3) N. S. Simmons, C. Cohen, A. G. Szent-Györgyi, D. B. Wetlaufer and E. R. Blout, *J. Am. Chem. Soc.*, **83**, 4766 (1961).

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

(5) W. Moffitt, D. D. Fitts and J. G. Kirkwood, *Proc. Natl. Acad. Sci. U. S.*, **43**, 723 (1957), and preceding papers; but see also J. A. Schellman and P. Oriel, *J. Chem. Phys.*, in press. We thank Dr. Schellman for making a preprint of his manuscript available.

(6) With identical reference and sample solutions, a rotational band causes sinusoidal oscillations in the transmission spectrum. Their envelope and phase are directly related to the magnitude and sign of the dichroism, respectively, for the small oscillations observed (0.02 to 3% T), provided adequately narrow slit-widths are maintained.

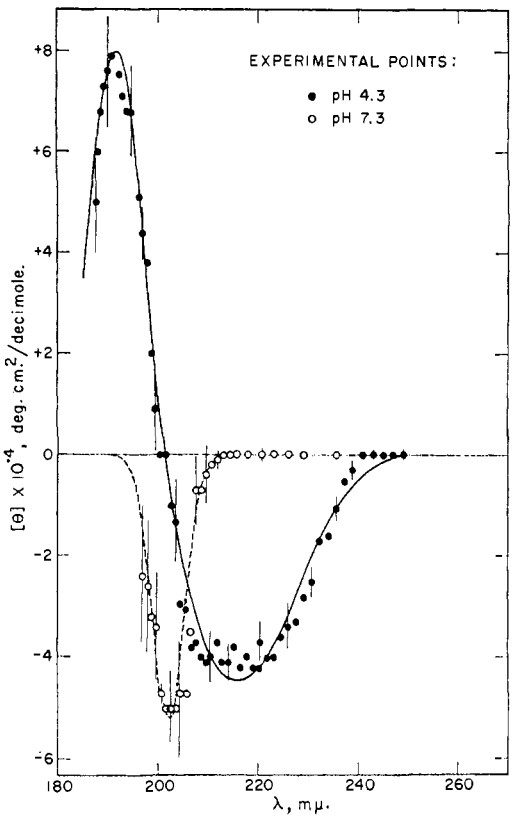

Fig. 1.—The circular dichroism of poly-L-glutamic acid in 0.1 *M* NaF at concentrations from 0.03 to 0.4%, determined spectrophotometrically.[8] The curves are $8.5 \times 10^4 \exp\{-(\lambda - 192)^2/(6.5)^2\} - 4.5 \times 10^4 \exp\{-(\lambda - 216)^2/(16)^2\}$ for the helix and $-5.2 \times 10^4 \exp\{-(\lambda - 202)^2/(4.5)^2\}$ for the coil. No refractive index corrections were made. Standard deviations of several determinations are shown at typical points.

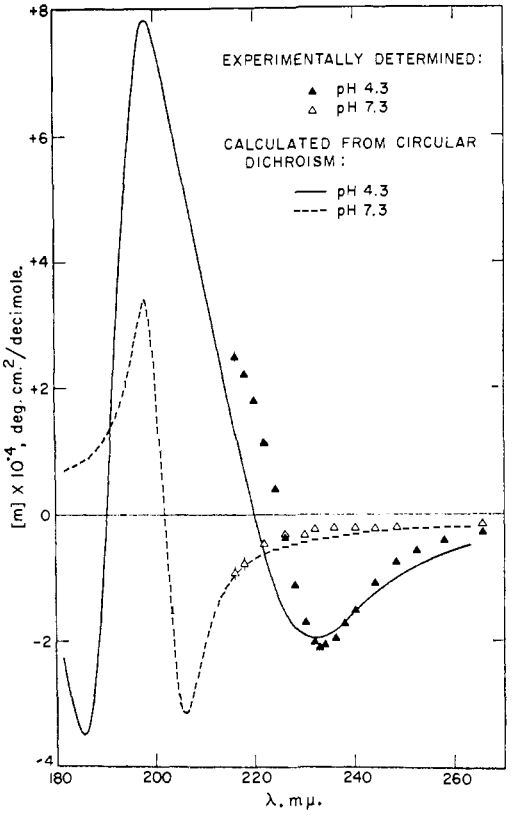

Fig. 2.—The calculated and observed mean residue rotation of poly-L-glutamic acid in 0.1 *M* NaF at concentrations from 0.03 to 0.4%, determined spectrophotometrically.[8] No refractive index corrections were made. Standard deviations are shown at typical points.

ments on testosterone were found to be in good agreement with published curves.[7]

The circular dichroism, expressed as the molecular ellipticity, of poly-L-glutamic acid in the helical and disordered forms is shown in Fig. 1. The lines are the sum of Gaussian curves $[\theta_K^0]\exp\{-(\lambda - \lambda_K)^2/(\Delta_K)^2\}$ chosen as a reasonable fit of the data.[8] Thus the helical form may be characterized by two bands centered at about 192 and 216–220 mμ with strengths $+3.6 \times 10^{-39}$ and -4.1×10^{-39} erg. cm.³ rad, respectively. The disordered form exhibits one band at 202 mμ with strength -1.4×10^{-39}.

From such curves the rotatory dispersion (as $[m]$, the mean residue rotation) can be calculated for each Gaussian with the Kronig–Kramers transform[9,10]

$$[m_K] = \frac{2[\theta_K^0]}{\sqrt{\pi}} \left\{ \int_0^{\frac{\lambda - \lambda_K}{\Delta_K}} e^{x^2} dx - \frac{\Delta_K}{\lambda + \lambda_K} \right\}$$

Such transforms generate the Cotton effects shown in Fig. 2. For comparison, direct measurements of the rotatory dispersion were made with a Rudolph 200S spectropolarimeter utilizing a mercury–xenon lamp. It is seen that the agreement is satisfactory to 212 mμ except for the 5 mμ difference in crossover points near 220 mμ. Evaluation of the parameter b_0 using 212 mμ for λ_0 and 1.33 for n yields −430 from the dichroic data and −690 from the dispersion data. The agreement, while not good, nevertheless permits one, in view of the neglect of internal field corrections, to conclude that the dichroic bands observed account for most of the complexity of the dispersion, as measured by b_0.

Circular dichroism may also be correlated with absorption spectra.[10] For the helical case, the breadth and location of the negative band prevent a direct assessment of the relative roles of the 206 mμ $\pi \rightarrow \pi^*$ and the $n \rightarrow \pi^*$ transitions. However, the positive band is clearly associated with the 191 mμ transition as is the negative band of the randomly coiled form with its 192 mμ transition.

Circular dichroic measurements also were made on poly-L-lysine and poly-γ-benzyl-L-glutamate (in dioxane, to 213 mμ); their helical forms exhibited dichroism very similar to that of helical poly-L-glutamic acid. Conversely, poly-L-lysine at pH 7 resembled randomly coiled poly-L-glutamic acid. Native metmyoglobin showed a rotational strength $^2/_3$ that of a fully helical polypeptide. Thus, the behavior shown in Fig. 1 appears to be

(7) L. Velluz and M. Legrand, *Angew. Chem.*, **73**, 603 (1961).
(8) K. Rosenheck and P. Doty, *Proc. Natl. Acad. Sci. U. S.*, **47**, 1775 (1961).
(9) A. Moscowitz, "Optical Rotatory Dispersion" (C. Djerassi, ed.), McGraw-Hill Book Co., New York, N. Y., 1960. Chapt. 12.
(10) W. Moffitt and A. Moscowitz, *J. Chem. Phys.*, **30**, 648 (1959).

a general characteristic of the two conformations of the polypeptide chain.

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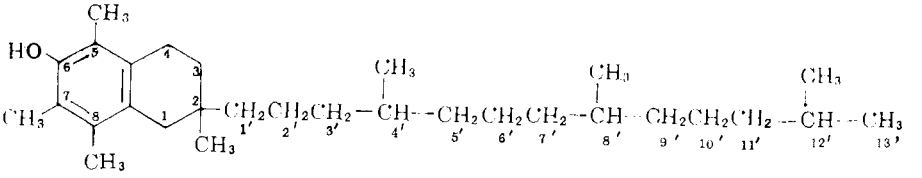
ISOLATION OF AN *l*-EPIMER OF NATURAL *d*-α-TOCOPHEROL[1]

Sir:

We wish to report the fractionation into diasteromers of synthetic α-tocopherol by means of a complex with piperazine and the isolation in pure form of an *l*-epimer of natural *d*-α-tocopherol. The α-tocopherol was prepared by reaction of natural phytol with trimethylhydroquinone. The physical properties of the *l*-epimer are reported here and its biopotency will be reported separately by Ames and Ludwig of this Laboratory. The biopotency was found to be substantially lower than that of natural *d*-α-tocopherol.

The fractionation procedure also gave a pure *d*-epimer whose properties served to identify it with natural *d*-α-tocopherol.

The synthesis of α-tocopherol by condensation of natural phytol with trimethylhydroquinone gives an epimeric mixture at the 2-position of the chroman ring. Since natural phytol contains two centers of optical activity with D-configurations,[2] the α-tocopherol so synthesized can be designated as 2-*dl*,4′D,8′D-α-tocopherol. The *l*-epimer isolated is more precisely designated as 2-*l*,4′D,8′D-α-tocopherol; the *d*-epimer isolated is correspondingly 2-*d*,4′D,8′D-α-tocopherol. The identification of the *d*-epimer with natural *d*-α-tocopherol provides evidence, heretofore lacking, that the latter has the same configuration at the asymmetric carbon atoms in the side chain as natural phytol.



The epimers were separated by repeated fractional crystallization of a solid complex, which we found that α-tocopherol forms with piperazine. This complex, which appears to be a coördination complex of two molecules of tocopherol with one of piperazine, is prepared readily by dissolving the components in approximately ten volumes of acetone and cooling the solution to effect crystallization. The tocopherol can be recovered by dissolving the complex in petroleum ether and washing with water to remove the piperazine.

The first crop of piperazine–tocopherol complex separated from a cooled (−20°) solution of the synthetic α-tocopherol (100 g., 1.0 mole proportion) and piperazine (2.5 g., 0.25 mole proportion) in acetone (200 ml.). The crystals so obtained contained a high proportion of the *d*-epimer (ratio *d*:*l* = 70:30), and after five recrystallizations yielded the pure *d*-epimer.

The original filtrate was again treated with piperazine to remove a second crop of complex enriched in *d*-epimer (ratio *d*:*l* = 61:39). The filtrate from this second crop now contained a preponderance of the *l*-epimer (ratio *l*:*d* = 59:41).

The *l*-enriched filtrate was further fractionated to obtain pure *l*-epimer. The fractionation was accomplished by repeated treatments of the recovered tocopherol with fresh portions of piperazine to precipitate the complex. The *l*-epimer was concentrated each time in the crystallized fraction, apparently because it was present in higher concentration. After seven fractionations we obtained 2-*l*,4′D,8′D-α-tocopherol, optically pure by our criteria. The yield was 1.7% without reprocessing of intermediate filtrate fractions.

TABLE I

OPTICAL ROTATION DATA

	2-*l*,4′D,8′D-α-tocopherol	2-*d*,4′D,8′D-α-tocopherol	Natural *d*-α-tocopherol
(A) Tocopherols			
$[\alpha]^{25}D$ (EtOH)	+0.36°	+0.58°	+0.65°
$[\alpha]^{25}D$ of $K_3Fe(CN)_6$ oxidation product (iso-octane)	−24.0°	+25.7°	+27.5°
(B) Acetates			
M.p., °C.	23	28[a]	28[b]
$[\alpha]^{25}D$ (EtOH)	−2.0°	+3.2°	+3.2°
(C) Acid Succinates			
M.p., °C.	51	78[c]	78[d]
$[\alpha]^{25}D$ (EtOH)	−2.9°	+3.8°	+3.8°

[a,b] Mixed melting point 28°. [c,d] Mixed melting point 78°.

The progress of the fractionation of the diasteromers was followed by the optical rotation of the fractions after oxidation. The α-tocopherol was recovered from the piperazine complex, oxidized with alkaline potassium ferricyanide and the optical rotation of the oxidized product was measured (Rudolph polarimeter, Model 70). The procedure is based on our finding[3] that the compound formed by oxidizing natural *d*-α-tocopherol with potassium ferricyanide has a relatively high specific rotation ($[\alpha]^{25}D$ +27.5°). When repeated precipitations of the complex produced α-tocopherol

(1) Communication No. 292 from the Research Laboratories of Distillation Products Industries, Division of Eastman Kodak Company, Rochester 3, New York.

(2) J. W. K. Burrell, L. M. Jackman, and B. C. L. Weedon, *Proc. Chem. Soc.*, 263 (1959).

(3) D. R. Nelan and C. D. Robeson, *Nature*, **193**, 477 (1962).