Separation of pure **7**, mp 232–233° dec, was achieved by fractional crystallization from ethanol. Treatment of **7** with thionyl chloride in THF in the presence of sodium carbonate and a catalytic amount of dimethylformamide produced the corresponding acid chloride which, by reaction with diazomethane, led to the diazo ketone **8**. Without isolation, **8** was subjected to copper-catalyzed thermolysis in cyclohexane to furnish **9** as white crystals, mp 196–197° dec (the overall yield from **7** to **9** was *ca.* 50%). Aromatization of **9** could be carried out with excess DDQ in refluxing chlorobenzene, affording **10** as orange needles (THF), mp 282–284° dec, in 45% yield.

The conversion of **10** to **11** by a Favorskii rearrangement smoothly occurred when **10** was heated with 20% KOH in methanol–water (50:50) at 90° (1 hr). Compound **11**, obtained as red cubes, mp 238–239° (THF), in 95% yield, was best characterized as its methyl ester, mp 153–154°: nmr ($CDCl_3$) multiplet at τ 1.55–2.30 (H_2–H_5 and H_9–H_{12}) and three singlets at 1.98 (H_7, H_{14}), 7.30 (methyl protons), and 11.69 (H_{16}).

Not too surprisingly, the decarboxylation of **11** proved to be the most demanding step. As conventional thermal methods failed to generate **3**, photochemical reactions were envisaged. Our attention chiefly focused on Barton's[9] photolysis of *S*-acyl xanthates, a reaction that in general leads to *S*-alkyl xanthates. Since the *S*-alkyl xanthates on hydrolysis afford thiols, the decarboxylation problem would in theory then be reduced to the task of desulfurization.

The *S*-acyl xanthate **13**, prepared from **12** and sodium *O*-ethyl xanthate, rapidly decarbonylated upon irradiation in benzene with a high-pressure mercury lamp (Philips HPK 125). Interestingly, the product obtained was not the anticipated *S*-alkyl xanthate but rather the desired hydrocarbon **3** (30% yield). Execution of the decarbonylation reaction in the presence of tri-*n*-butyltin hydride afforded **3** in yields as high as 60%. 1,6:8,13-Ethanediylidene[14]annulene (**3**) thus produced forms scarlet red plates, mp 118–119° (methanol), and is a perfectly stable compound.

The 100-MHz nmr spectrum of **3** (0.35 *M* ($CDCl_3$); TMS), consisting of an AA′BB′ system at τ_A 1.83 (H_2, H_5 and H_9, H_{12}) and τ_B 2.18 (H_3, H_4 and H_{10}, H_{11}) ($J_{23} = 9.42$, $J_{24} = 0.47$, $J_{25} = 1.17$, and $J_{34} = 9.72$ Hz) and two singlets at τ 2.00 (H_7, H_{14}) and 11.82 (H_{15}, H_{16}), rigorously proves the assigned structure and, moreover, in agreement with the assumptions based on molecular models, indicates a noticeably stronger ring current effect for the compound than is observed for the higher homologs **4** and **5**. The uv spectrum of **3** [λ_{max} (cyclohexane) 308 (ϵ 202, 500), 339 (25, 100), 381 (10, 900), 398 sh (6000), 472 (350), and 551 nm (3700)] is similar to those of **4** and **5**, but shows a bathochromic shift and increased intensity for the band of longest wavelength.

A most remarkable chemical feature of **3** is its resistance toward dehydrogenation under a variety of conditions. Thus, the hydrocarbon remains essentially unaffected when treated with Pd/$BaSO_4$ in boiling decalin (*ca.* 200°!) for 2 hr, even though the anticipated product, **1**, has been postulated to be a stable aromatic molecule.[7a]

The relationship of **3** and its hypothetical trans isomer to Boekelheide's[10] *cis*- and *trans*-15,16-dihydropyrenes will be discussed in a forthcoming publication.

[9] D. H. R. Barton, M. V. George, and M. Tomoeda, *J. Chem. Soc.*, 1967 (1962).

[10] For a review on 15,16-dihydropyrenes, see V. Boekelheide, *Proc. Robert A. Welch Found. Conf. Chem. Res.*, 12, 83 (1968); see also, R. H. Mitchell and V. Boekelheide, *Chem. Commun.*, 1555 (1970).

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Circular Dichroism of Polypeptide Solutions in the Vacuum Ultraviolet

Sir:

Previous measurements of the circular dichroism (CD) of polypeptides have been limited to the wavelength region above 183 nm.[1,2] It is important to make measurements below this wavelength cutoff of commercial instruments, because theoretical calculations of the CD spectrum predict a large negative peak near 180 nm.[3,4] The predicted peak is assigned to the negative lobe of a CD band which has the shape of the derivative of an absorption curve.[5] This type of CD band is central to the correct interpretation and understanding of the CD of helical polymers.[6,7]

Figure 1 shows the measured CD of poly-L-glutamic acid as a helix (pH 4.5) and coil (pH 8), and of *N*-acetyl-L-alanine-*N*′-methylamide. The molecules were dissolved in water and adjusted to the required pH with NaOH; no other salt was added. All measurements were made on a vacuum ultraviolet CD spectrometer described elsewhere.[8] The spectrometer was calibrated using a value of $\epsilon_L - \epsilon_R = 2.20$ at 290.5 nm for *d*-10-camphorsulfonic acid in water.[1] Samples were prepared by putting 1 drop of solution with a concentration of about 5 mg/ml between polished, clean CaF_2 windows with no spacer. Windows were carefully chosen so that they mated with only a single fringe over most of their area. The sample thickness was about 1–10 μ and was controlled by the size of the drop put on the CaF_2 plate. Different path lengths were used for different wavelength regions, but the total absorbance of the cell, solvent, and sample was kept below 1.0 for all measurements. The sample thickness was determined from the known CD of the molecules in the near-ultraviolet. The helix CD at 190 nm and the coil CD at 195 nm were chosen as reference points.[9]

Instead of a negative peak below 180 nm, a positive shoulder is seen for the helix in Figure 1. This must be caused by a transition not previously considered in the theoretical analysis. Cassim and Yang[1] had inferred the absence of a negative peak near 180 nm from a careful comparison of their measured CD and ORD above 183 nm. They concluded that either the theory of the CD of polypeptides was in error, or an unexpected new band was cancelling the predicted trough.

[1] J. Y. Cassim and J. T. Yang, *Biopolymers*, 9, 1475 (1970).

[2] J. R. Parrish, Jr., and E. R. Blout, *ibid.*, 10, 1491 (1971).

[3] R. W. Woody and I. Tinoco, Jr., *J. Chem. Phys.*, 46, 4927 (1967).

[4] R. W. Woody, *ibid.*, 49, 4797 (1968).

[5] I. Tinoco, Jr., *J. Amer. Chem. Soc.*, 86, 297 (1964).

[6] W. Moffitt, *J. Chem. Phys.*, 25, 467 (1956).

[7] W. Moffitt, D. D. Fitts, and J. G. Kirkwood, *Proc. Nat. Acad. Sci. U. S.*, 43, 1046 (1957).

[8] W. C. Johnson, Jr., *Rev. Sci. Instrum.*, 42, 1283 (1971).

[9] A. J. Adler, R. Hoving, J. Potter, M. Wells, and G. D. Fasman, *J. Amer. Chem. Soc.*, 90, 4736 (1968).

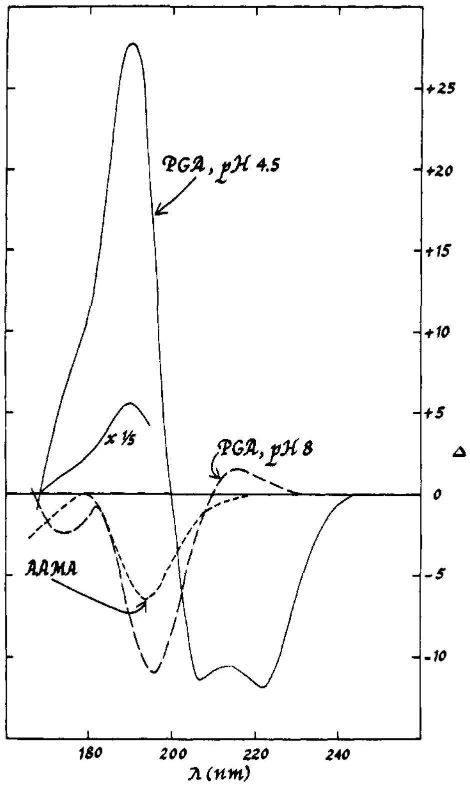

Figure 1. Measured circular dichroism for aqueous solutions of polyglutamic acid as a helix (PGA, pH 4.5) and coil (PGA, pH 8), and of N-acetyl-L-alanine-N'-methylamide (AAMA).

They favored the former conclusion. We have never doubted the existence of a CD band with special characteristics for perpendicular polarized transitions in helices. Indeed, there has been recent new evidence for it. The prediction that this band should occur only for light incident along the helix axis has been verified for flow-oriented DNA.[10] New theoretical derivations of the optical activity of long helices[11,12] have discussed how this band arises. For circularly polarized light incident along the helix axis of a long helix, conservation of angular momentum for the light-molecule system must be considered. This leads to a very small splitting into two bands which gives the characteristic derivative shape for the CD band.

Unequivocal assignment of the new CD peak seen as a shoulder in the helix CD at about 175 nm is difficult. Only suggestive evidence can be given. No intense absorption band is seen in this wavelength region in polypeptide films.[13,14] However, a negative CD peak is present in the coil CD spectrum at 175 nm. A transition in the polypeptide which is mainly magnetically allowed is consistent with these results. It would have weak absorption and its contribution to the CD would be very dependent on the conformation of the polymer. An n → σ^* centered on the carbonyl oxygen fits the requirements; it was placed at 150 nm in the original calculations.[3] Further evidence for the assignment is the CD spectrum of N-acetyl-L-alanine-N'-methylamide (AAMA). The n → π^* in AAMA seems to be blue shifted relative to the n → π^* near 220 nm in polypeptides. Similarly, there is a blue shift near 175 nm, although the π → π^* band near 195 nm is unchanged. This is consistent with the transitions near 220 and 175 nm having the same origin, nonbonding electrons on oxygen.

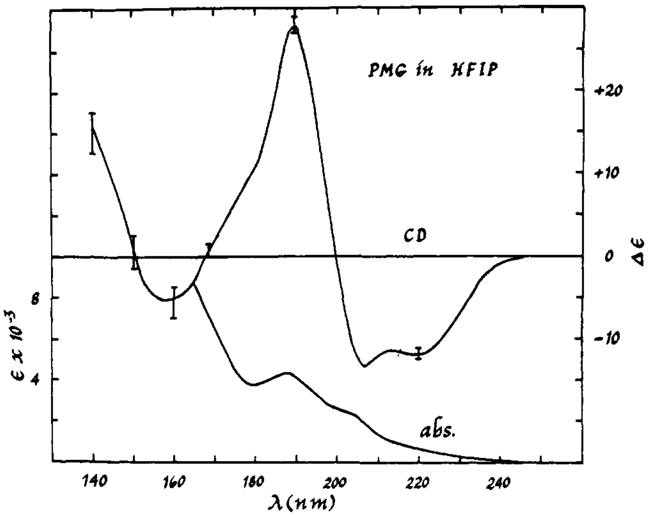

Figure 2. Measured circular dichroism and absorption for poly-γ-methyl glutamate in hexafluoro-2-propanol.

Figure 2 shows the CD spectrum of poly-γ-methyl L-glutamate (PMG) in hexafluoro-2-propanol (HFIP). This spectrum indicates that the predicted negative peak is not lost because of orientation effects. Our polypeptide has a molecular weight of about 250,000 or an α-helix length of approximately 0.3 μ. Thus in our thin cell work, the path length was only three to ten times the α-helix length. However, this spectrum was calibrated using a commercially available cylindrical quartz cell with a path length measured interferometrically to be 63 μ. This cell is 200 times the length of the helix, and the spectrum recorded to 166 nm is identical with that measured using the thin cell techniques. It shows the shoulder at 175 nm and the absence of the predicted negative band.

The three extrema above 180 nm in Figure 2 have the same position and shape as those measured earlier,[2] but the magnitudes are about 25% higher. Additional transitions are seen as a shoulder at 175 nm, a negative peak at 159 nm, and the indication of a maximum below 140 nm. Transitions near 160 and 140 nm have been seen in absorption spectra of polypeptide films.[13,14] These CD measurements cannot provide help in their assignment without detailed calculations.

The instrumentation and methods for measuring the CD of aqueous solutions to 167 nm and other solutions to 140 nm should prove useful in many other applications for biologically interesting molecules.

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[10] S. Y. Wooley and G. Holzwarth, *J. Amer. Chem. Soc.*, **93**, 4066 (1971).

[11] F. M. Loxsom, *J. Chem. Phys.*, **51**, 4899 (1969).

[12] C. W. Deutsche, *ibid.*, **52**, 3703 (1970).

[13] J. Brahms, J. Pilet, H. Damany, and V. Chandrasekharan, *Proc. Nat. Acad. Sci. U. S.*, **60**, 1130 (1968).

[14] J. L. Bensing and E. S. Pysh, *Macromolecules*, **4**, 659 (1971).