electron-withdrawing power of the nitro or sulfonyl groups than cyano or ester groups, as measured by σ values,[23,23a] indicates the frontier orbitals of the former will be at lower energy than those of the latter.

The results reported here are in variance with those predicted by a postulated diradical mechanism,[24] and, furthermore, incursion of a dipolar intermediate mechanism seems unlikely in light of the negligible effect of solvent polarity changes on the rates of these reactions,[25] and the increase, rather than decrease, of 5-substituted adducts with increasing solvent polarity in the closely related nitrile oxide–methyl propiolate reactions.[26]

Acknowledgment. Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for support of this research.

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[23] E. M. Kosower, "An Introduction to Physical Organic Chemistry," Wiley, New York, N. Y., 1968.

[23a] Note Added in Proof: Although photoelectron spectra (Perkin-Elmer PS-18) of nitroethylene and phenyl vinyl sulfone are insufficiently resolved for definitive assignments, vertical IP values of 11.38 and $\sim$11.4 eV, respectively, are probably due to alkene π ionizations. These spectra were measured by J. Meeks and L. Lambert in these laboratories.

[24] R. A. Firestone, *J. Org. Chem.*, 33, 2285 (1968); 37, 2181 (1972).

[25] J. Sims, unpublished results with **1a** and cyanoacetylene indicate solvent effects on reaction rates nearly identical with those found with **1a** and ethyl acrylate.[10]

[26] M. Christl, and R. Huisgen, *Chem., Ber.* in press.

[27] On leave from Chipola Junior College, Marianna, Florida, 1972–1973.

[28] Camille and Henry Dreyfus Foundation Teacher–Scholar Grant Recipient, 1972–1977.

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Minimum Chain Length Required for the Observation of a Temperature-Sensitive, Salt-Sensitive, Positive Circular Dichroism Band in Derivatives of L-Alanine[1]

Sir:

The conformational properties of ionized homopolymers in aqueous solvents are highly dependent upon ionic strength.[2] Certain salts can also alter the stability of the ordered conformations formed by proteins.[3] The effects upon the conformational properties of proteins are not due simply to alterations in ionic strength because the result observed depends upon the type of salt used[3] and similar results can be observed in homopolypeptides which do not contain ionizable side chains.[4]

Fully ionized homopolypeptides, which have been widely used as models for polypeptides in a random coil conformation,[5] exhibit a small positive circular dichroism band near 216 nm[6] whose intensity is decreased by increasing either salt concentration or temperature.[4,7–9] This observation has been the basis for proposing the existence of a new ordered structure, the "extended helix," which is presumed to be stabilized by electrostatic interaction of the charged side chains.[7–11] According to this proposal,[7–11] the random coil exhibits only negative circular dichroism above 200 nm, and the small positive band observed near 216 nm arises from the "extended helix." Evidence against the existence of an ordered structure stabilized by the electrostatic interaction of charged side chains has been provided by comparison of the optical activity[4,12] and unperturbed dimensions[13] of ionized poly(L-glutamic acid) and an un-ionized random-coil derivative, poly(N^5-ω-hydroxyethyl-L-glutamine).

The effects of solvent composition and temperature upon the circular dichroism exhibited by a variety of low molecular weight derivatives of L-alanine are being investigated in order to clarify the mechanism of the interaction of polypeptides with salts. A few of the significant characteristics observed with several of these compounds are presented here. The circular dichroism spectra were obtained using a Jasco J-20 spectropolarimeter calibrated with *d*-10-camphorsulfonic acid.[14] The results for the small derivatives of L-alanine are presented as the mean ellipticity per carbonyl group[15] in order to facilitate comparison to the mean residue ellipticity reported for polypeptides.

The circular dichroism of Ac-Ala-OMe, Ac-Ala-Ala-OMe, and Ac-Ala-Ala-Ala-OMe[16] in water at 15°, shown in Figure 1, exhibit an extremely small negative band at 231–234 nm, a weak positive band at 208–214 nm, and a strong negative band at or below 190 nm. Increasing the chain length through this series increases the intensity of the negative circular dichroism observed below 200 nm and produces a slight decrease in intensity and red shift for the maximum positive circular dichroism. The final member of this series would be random-coil poly(L-alanine) of high molecular weight, which cannot be studied in water due to its insolubility.[17] A useful substitute with a $-CH_2R$ side chain is poly(N^5-ω-hydroxyethyl-L-glutamine), an un-ionized polypeptide which assumes a random coil conformation in water.[13,18] Extension of the trends observed, in water, in the circular dichroism of the series Ac-Ala-OMe, Ac-Ala-Ala-OMe, Ac-Ala-Ala-Ala-OMe leads to the circular dichroism pattern observed in this solvent with poly(N^5-ω-hydroxyethyl-L-glutamine).[4,12]

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[1] This work was supported by Grant GB-36055 from the National Science Foundation.

[2] P. J. Flory, "Principles of Polymer Chemistry," Cornell University Press, Ithaca, N. Y., 1953, p 635.

[3] P. H. von Hippel and T. Schleich in "Structure and Stability of Biological Macromolecules," S. N. Timasheff and G. D. Fasman, Ed., Marcel Dekker, New York, N. Y., 1969, p 417.

[4] W. L. Mattice, J.-T. Lo, and L. Mandelkern, *Macromolecules*, **5**, 729 (1972).

[5] G. D. Fasman in "Poly-α-amino Acids," G. D. Fasman, Ed., Marcel Dekker, New York, N. Y., 1967, p 499.

[6] S. Beychok in ref 5, p 293.

[7] M. L. Tiffany and S. Krimm, *Biopolymers*, **6**, 1379 (1968).

[8] M. L. Tiffany and S. Krimm, *ibid.*, **8**, 347 (1969).

[9] S. Krimm, J. E. Mark, and M. L. Tiffany, *ibid.*, **8**, 695 (1969).

[10] S. Krimm and J. E. Mark, *Proc. Nat. Acad. Sci. U. S.*, **60**, 1122 (1968).

[11] W. A. Hiltner, A. J. Hopfinger, and A. G. Walton, *J. Amer. Chem. Soc.*, 94, 4324 (1972).

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

[13] W. L. Mattice and J.-T. Lo, *Macromolecules*, **5**, 734 (1972).

[14] J. Y. Cassim and J. T. Yang, *Biochemistry*, **8**, 1947 (1969).

[15] The reported circular dichroism therefore represents the ellipticity per mole divided by the number of chromophores contributing to the ellipticity.

[16] The abbreviations are those recommended by the IUPAC–IUB Commision of Biochemical Nomenclature, *Biochemistry*, **11**, 1726 (1972).

[17] E. Brand, B. Erlanger, and H. Sachs, *J. Amer. Chem. Soc.*, 74, 1849 (1952).

[18] N. Lotan, A. Yaron, and A. Berger, *Biopolymers*, 4, 365 (1966).

The spectra obtained with the three alanine derivatives in water at 75° are also shown in Figure 1. The rate of change of the ellipticity at a particular wavelength, for a given compound, with temperature is nearly constant over this temperature range. In each case the positive circular dichroism is reduced by increasing the temperature. At 75° a small positive circular dichroism is still observed with Ac-Ala-OMe, but no positive circular dichroism is evident with Ac-Ala-Ala-OMe or Ac-Ala-Ala-Ala-OMe. The temperature effect at 216 nm (the location of the maximum positive circular dichroism observed with poly(N^5-ω-hydroxyethyl-L-glutamine) in water at 30°)[4] is given in Table I along with the results obtained for an ionized and an un-ionized homopolypeptide.[4] It is apparent that the temperature effect observed with random coil polypeptides also occurs in these small derivatives of alanine.

Table I. Temperature Effect upon the Circular Dichroism Observed in Water at 216 nm

Compound	d[θ]/dT, deg cm^2/dmol °C
Ac-Ala-OMe	−15
Ac-Ala-Ala-OMe	−36
Ac-Ala-Ala-Ala-OMe	−50
Poly(N^5-ω-hydroxyethyl-L-glutamine)[a]	−49
Poly(L-glutamic acid)[a,b]	−64

[a] Reference 4. [b] pH 6.55, ionic strength adjusted to 0.025 M with potassium chloride.

The spectral properties of an internal L-alanine residue in water can be estimated by applying the procedure described by Doyle, *et al.*[19] The *molar* ellipticities are required for this purpose. They can be obtained from the spectra in Figure 1 by multiplying in each case by the number of carbonyl groups per compound. Subtraction of the molar ellipticity of Ac-Ala-OMe from that of Ac-Ala-Ala-OMe in water at 15° yields a positive circular dichroism of 2500 deg cm^2/dmol at 214 nm. A similar subtraction for the data at 75° gives $[\theta]_{214} = -2500$ deg cm^2/dmol. Subtraction of the molar ellipticity of Ac-Ala-Ala-OMe from that of Ac-Ala-Ala-Ala-OMe at 15° yields a positive circular dichroism of 500 deg cm^2/dmol at 217 nm, and $[\theta]_{217} = -5000$ deg cm^2/dmol at 75°. While the results for these two comparisons are not quantitatively identical, they both show that an internal L-alanine residue will provide a positive circular dichroism at 214–217 nm at 15°, and that the circular dichroism in this spectral region will become negative upon heating.

The circular dichroism spectra observed with the three alanine derivatives in 4 M sodium perchlorate at 15° are shown in Figure 1. The isothermal addition of 4 M sodium perchlorate reduces the positive circular dichroism exhibited by Ac-Ala-OMe. No positive circular dichroism is observed with Ac-Ala-Ala-OMe or Ac-Ala-Ala-Ala-OMe in 4 M sodium perchlorate at 15°. The effect of 4 M calcium chloride upon Ac-Ala-Ala-Ala-OMe is similar to that of 4 M sodium perchlorate. The effect of sodium perchlorate is slightly larger than that of calcium chloride in the case of the two smaller alanine derivatives. Therefore, the salt effects observed in the circular dichroism of charged and uncharged polypeptides in water[4,7–9] also manifest themselves in these small derivatives of alanine.

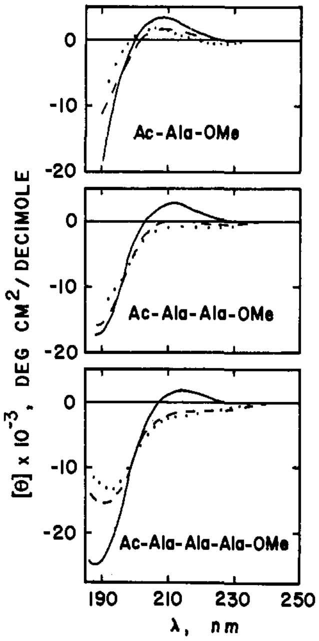

Figure 1. Circular dichroism of Ac-Ala-OMe, Ac-Ala-Ala-OMe, and Ac-Ala-Ala-Ala-OMe in water at 15° (solid line) and 75° (dashed line) and in 4 M sodium perchlorate at 15° (dotted line).

It has been reported that only negative circular dichroism is observed above 190 nm for Ac-Ala-NHMe in water at 27.0°[20] and at an unspecified temperature, presumably ambient.[21] Measurement of the circular dichroism in water over the range 5–75° shows that for Ac-Ala-NHMe also d[θ]/dT near 216 nm is negative. A positive circular dichroism is observed near 218 nm with Ac-Ala-NHMe in water at 15°. No positive circular dichroism is observed at this temperature in 4 M sodium perchlorate or 4 M calcium chloride.

The conformational map for Ac-Ala-NHMe[22] contains several minima which are within 2 kcal/mol of each other. A Boltzmann distribution over this conformational map[22] would include a contribution from the combination of ϕ[23] and ψ attributed to the "extended helix,"[10,11] but would also include contributions from other ϕ and ψ as well. The effect on the conformational map of substituting an ester for an amide is to increase the freedom of rotation about ψ, as can be seen by comparing the internal dipeptide in poly(L-alanine)[24] with the internal diester in poly(L-lactic acid)[25] or by comparing Ac-Pro-NHMe and Ac-Pro-OMe.[26] The conformational map for Ac-Ala-

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(19) B. B. Doyle, W. Traub, G. P. Lorenzi, and E. R. Blout, *Biochemistry*, **10**, 3052 (1971).

(20) J. R. Cann, *ibid.*, **11**, 2654 (1972).

(21) W. C. Johnson, Jr., and I. Tinoco, Jr., *J. Amer. Chem. Soc.*, **94**, 4389 (1972).

(22) P. K. Ponnuswamy, P. K. Warme, and H. A. Scheraga, *Proc. Nat. Acad. Sci. U. S.*, 70, 830 (1973).

(23) IUPAC-IUB Commission on Biochemical Nomenclature, *Biochemistry*, **9**, 3471 (1970); *J. Mol. Biol.*, **52**, 1 (1970).

(24) D. A. Brant, W. G. Miller, and P. J. Flory, *J. Mol. Biol.*, **23**, 47 (1967).

(25) D. A. Brant, A. E. Tonelli, and P. J. Flory, *Macromolecules*, **2**, 228 (1969).

(26) V. Madison and J. Schellman, *Biopolymers*, **9**, 511 (1970).

OMe[27] indicates much freer rotation about ϕ and ψ in this molecule than in Ac-Ala-NHMe. A Boltzmann distribution over the conformational map for Ac-Ala-OMe would give less weight to the combination of ϕ and ψ attributed to the "extended helix"[10,11] than is the case in Ac-Ala-NHMe.

The results reported here demonstrate that a small positive circular dichroism, which is sensitive to temperature and to the presence of salts, can be observed in peptides where the side chain is simply a methyl group and in a structure as small as Ac-Ala-OMe or Ac-Ala-NHMe. These effects cannot arise from the electrostatic interaction of charged side chains nor can they be due to an "extended helix." The temperature effects on the circular dichroism must instead reflect an increasing population of the higher energy regions of the conformational maps as the temperature increases. Calcium chloride and sodium perchlorate, as well as certain other salts, are known to affect the conformational properties of a wide variety of polypeptides.[3] The basic effect, as detected by circular dichroism, is also operative in very small molecules and can perhaps more easily be understood by studying small peptides rather than polypeptides.

[27] W. L. Mattice, unpublished results.

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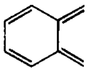
Photochemical Preparation of *o*-Xylylene from 1,4-Dihydrophthalazine in Rigid Glass[1]

Sir:

o-Xylylene (**1**) has evoked intense theoretical[2,3] and experimental[4-14] interest. While direct observations of substituted *o*-xylylenes of varying stabilities have succeeded,[6] evidence for **1** itself is only indirect (a stable metal complex is known[7]). The presence of **1** as an intermediate has been inferred from the nature of the products of trapping[8-10] or self-trapping reactions. The latter give **2**, **3**, **4**, and *o*-xylene. Some reactions which presumably proceed *via* **1** are oxidation[11] of **5** at 0° (**5** → **6** → **1**), thermal decompositions of **7**[9,10,12] and **8**,[13] and uv irradiation[14] of **9**.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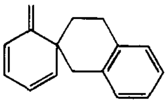
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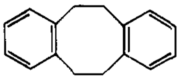
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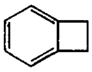
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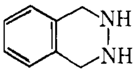

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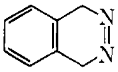

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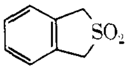

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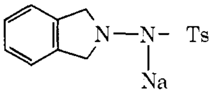

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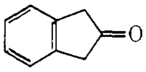

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Our interest in **1** was aroused by work on related hydrocarbons[15] and by studies of the electronic states of biradicals and of photochemical singlet–triplet reactivity differences.[16] This prompted us to search for biradicals whose lowest excited singlet (S_1) and triplet (T_1) states would have the same orbital occupancy; yet, S_1 could be shown to prefer a "tight" geometry and T_1 a "loose" one.[16]

In view of the reactivity of **1** matrix isolation techniques appeared appropriate. We now report a photochemical synthesis of **1** in rigid glass from the previously unreported **6** and some spectral properties of both. Pure **6** is a white powder and was prepared by oxidation of a basic (MeOK or KOH) methanolic solution of **5** at *ca.* $-90°$ with *t*-BuOCl, evaporation of methanol ($-78°$, 5×10^{-6} Torr), and chromatography of the residue on a silica gel column at $-80°$[17] (C_3H_8 − Me_2O): pmr in acetone-d_6 ($-70°$) singlets at τ 4.96 and 2.59, equal integrated intensities (*cf.* τ 5.15 for the methylene protons in 1,1′-diphenylazomethane[18] and τ 4.2 in 1,4-dihydronaphtho[1,8-*d*,*e*][1,2]diazepine[11]); uv in Et_2O ($-100°$) a broad band, λ_{max} 358 nm, ϵ_{max} $\sim 10^2$ (-N=N-), sharp peaks, λ_{max} 265 and 271 nm, ϵ_{max} $\sim 10^3$ (aromatic). Above $-40°$, the uv and pmr spectra of **6** disappeared, N_2 was evolved, and the known spectra[4] of **2** appeared (preliminary results at -40 to $-60°$ give first-order kinetics, $\Delta H^{\ddagger} = 15 \pm 2$ kcal/mol, $\Delta S^{\ddagger} = -7.5 \pm 3$ eu).

Irradiation (λ 254 nm or $\lambda > 345$ nm) of **6** at $-196°$ (glassy diethyl ether or EPA) destroyed its characteristic uv absorption and caused rapid formation of a new species (λ_{max} 373 nm, $\epsilon_{max} > 3000$). The Franck–Condon forbidden shape of the new band and the vibrational spacing (1400–1500 cm^{-1}) were strongly reminiscent of spectra of related *o*-quinomethides.[19] The photoproduct showed strong fluorescence (spectral shape independent of excitation wavelength, λ_{max} 456

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[2] J. Baudet, *J. Chim. Phys. Physicochim. Biol.*, **68**, 191 (1971).

[3] N. C. Baird, *J. Amer. Chem. Soc.*, **94**, 4941 (1972).

[4] L. A. Errede, *J. Amer. Chem. Soc.*, **83**, 949 (1961).

[5] W. Oppolzer, *Angew. Chem., Int. Ed. Engl.*, **11**, 1031 (1972); D. A. Ben-Efraim and F. Sondheimer, *Tetrahedron Lett.*, 313 (1963); F. R. Jensen, W. E. Coleman and A. J. Berlin, *Tetrahedron Lett.*, 15 (1962); L. A. Carpino, *J. Amer. Chem. Soc.*, **84**, 2196 (1962); K. Sisido, Y. Udo, and H. Nozaki, *J. Org. Chem.*, **26**, 584 (1961).

[6] For leading references see: L. Klasinc, E. Pop, N. Trinajstic, and J. V. Knop, *Tetrahedron*, **28**, 3465 (1972); G. M. Priestley and R. N. Warrener, *Tetrahedron Lett.*, 4295 (1972); K. Blatt and R. W. Hoffmann, *Justus Liebigs Ann. Chem.*, **760**, 114 (1972); U. E. Wiersum and W. J. Mijs, *J. Chem. Soc., Chem. Commun.*, 347 (1972); R. Bonnett and R. F. C. Brown, *J. Chem. Soc., Chem. Commun.*, 393 (1972); G. Quinkert, J. Palmowski, H.-P. Lorenz, W.-W. Wiersdorff, and M. Finke, *Angew. Chem., Int. Ed. Engl.*, **10**, 198 (1971); J. P. Anhalt, E. W. Friend, Jr., and E. H. White, *J. Org. Chem.*, 37, 1015 (1972).

[7] W. R. Roth and J. D. Meier, *Tetrahedron Lett.*, 2053 (1967).

[8] N. L. Bauld, F. R. Farr, and C.-S. Chang, *Tetrahedron Lett.*, 2443 (1972).

[9] K. Sisido, K. Tani, and H. Nozaki, *Tetrahedron*, **19**, 1323 (1963).

[10] M. P. Cava and A. A. Deana, *J. Amer. Chem. Soc.*, **81**, 4266 (1959).

[11] L. A. Carpino, *J. Amer. Chem. Soc.*, 85, 2144 (1963).

[12] J. R. du Manoir and J. F. King, *J. Chem. Soc., Chem. Commun.*, 541 (1972).

[13] W. Baker, J. F. W. McOmie, and D. R. Preston, *J. Chem. Soc.*, 2971 (1961).

[14] G. Quinkert, K. Opitz, W.-W. Wiersdorff, and J. Weinlich, *Tetrahedron Lett.*, 1863 (1963).

[15] (a) J. Kolc and J. Michl, *J. Amer. Chem. Soc.*, **92**, 4147 (1970); J. Michl and J. Kolc, *J. Amer. Chem. Soc.*, **92**, 4148 (1970); (b) J. Downing, V. Dvořák, J. Kolc, A. Manzara, and J. Michl, *Chem. Phys. Lett.*, **17**, 70 (1972).

[16] For leading references see: J. Michl, *Mol. Photochem.*, 4, 243, 257, 287 (1972); see also W. T. Borden and L. Salem, *J. Amer. Chem. Soc.*, **95**, 932 (1973).

[17] K. Hojo, R. T. Seidner, and S. Masamune, *J. Amer. Chem. Soc.*, 92, 6641 (1970).

[18] J. P. Freeman, *J. Org. Chem.*, **28**, 2508 (1963).

[19] V. Dvorák, J. Kolc, and J. Michl, *Tetrahedron Lett.*, 3443 (1972); L. Edwards, J. Kolc, and R. S. Becker, *Photochem. Photobiol.*, **13**, 423 (1971).