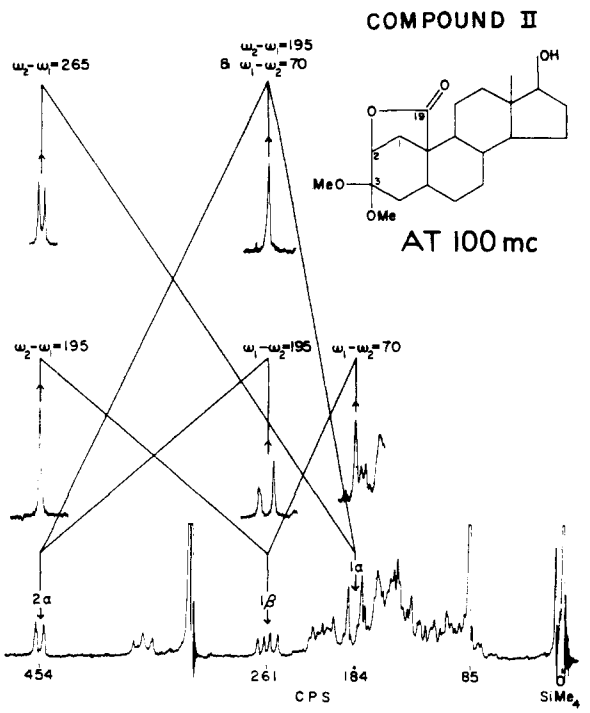

Fig. 1b.

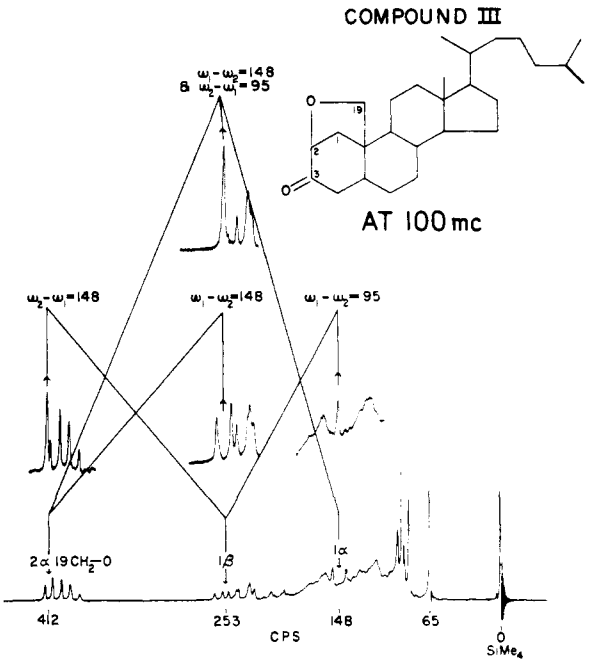

Fig. 1c.

Three principal regions are of interest. The signals at $\delta = 4.12$ in the spectrum of Compound III arise from the 2α-H spin coupled to the 1β-H as shown by n.m.d.r.; the 1α-H is virtually uncoupled from the 2α-H owing[6] to a dihedral angle

TABLE I

CHEMICAL SHIFT (P.P.M.) VALUES AND SPIN COUPLINGS (C.P.S.) FOR STEROID DERIVATIVES

Compound	2α-H	1β-H	1α-H	C.P.S. $J_{2\alpha \text{ and } 1\beta}$	C.P.S. $J_{2\alpha \text{ and } 1\alpha}$	C.P.S. $J_{1\alpha \text{ and } 1\beta}$
I	4.15	2.13	1.58	7	1	12
II	4.54	2.61	1.84	7	1	12
III	4.12	2.53	1.48	7	..	12

of approximately 90° (Courtauld models) between the atoms. In the case of Compounds I and II this proton appears as a pair of doublets at $\delta = 4.15$ and 4.54, respectively. Here the dihedral angle between 2α-H and 1α-H is somewhat greater than in III. A pair of doublets at $\delta = 2.13$, 2.61 and 2.53 in the spectra of Compounds I, II, and III, respectively, is due to the 1β-H spin-coupled to both the 1α-H and the 2α-H. This multiplet collapsed to a doublet on double irradiation at the 1α-H frequency, whereas a singlet resulted in the n.m.t.r. experiment.

The position of the 1α-H resonance was uncertain because of nearby signals from other protons. However, its location was uniquely determined by double irradiation at the frequency of the 2α-H, which caused the 1α-H doublet to degenerate to a singlet. The chemical shift between the 1α-H and the 1β-H in II and III is too large to be explained in terms of the axial and equatorial conformations of these protons, but this is not the case in I, in which $\delta\beta$ is of the expected magnitude for such a pair. These data indicate that the carbonyl group at C(19) or C(3) exerts a long range negative shielding effect on the equatorial 1β-H, and the axial 1α-H, in Compound II, but not on axial 1α-H in Compound III. This phenomenon is in harmony with the previously described[7] approximation of the long range shielding effect of the carbonyl group. In conventional steroids, the methyl protons at C(19) are located in an approximately geometrically equivalent position relative to the C(3) carbonyl group as the 1β-H in Compound III. And therefore the displacement of the resonance due to the C(19) protons in a 3-oxosteroid to lower field by 0.15 p.p.m.[8] can be explained in a similar way.

(7) L. M. Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon, New York, N. Y., 1959, p. 124.

(8) J. N. Shoolery and M. T. Rogers, *J. Am. Chem. Soc.*, **80**, 5121 (1958).

(9) M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959).

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RECEIVED SEPTEMBER 17, 1962

THE STABILIZATION TOWARD TEMPERATURE OF THE HELICAL CONFORMATION OF COPOLYPEPTIDES OF L-GLUTAMIC ACID AND L-LEUCINE: AN INVERSE TEMPERATURE EFFECT

Sir:

The investigation of the forces responsible for conformational stability of proteins and polypeptides has, in recent years, led to the hypothesis that hydrophobic forces play an important role in

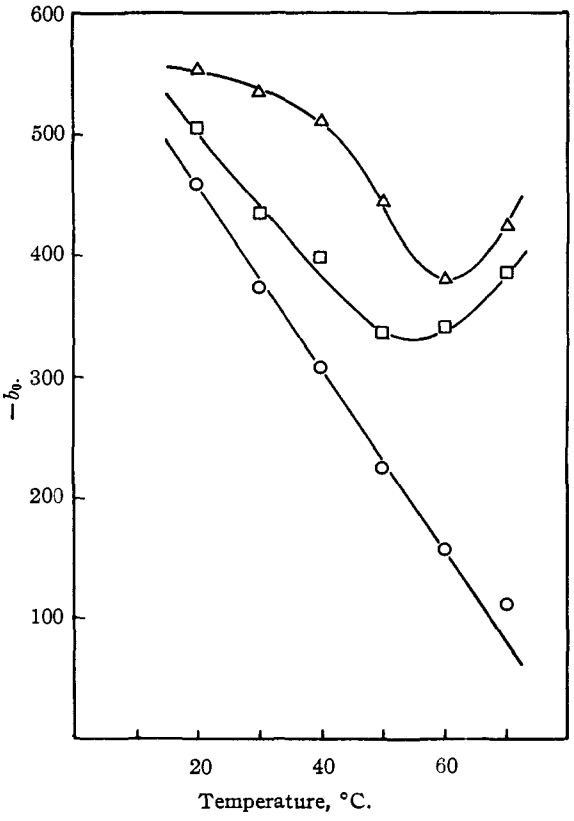

Fig. 1.—The b_0 values (from the Moffitt equation, λ_0 = 212 mμ, for the wave length range 365 to 589 mμ) as a function of temperature for poly-L-glutamic acid (c = 1.0%), ⊙; copoly-L-glutamic acid:L-leucine, 80:20 (c = 0.5%), ⊡; and copoly-L-glutamic acid:L-leucine, 75:25 (c = 0.5%), △; all polymers in 0.2 M NaCl, pH 4.88.

maintaining native structures.[1-10] The consequences of the interaction of non-polar side chains with each other and with the aqueous solvent[1-4] have been examined thermodynamically and it has been postulated that such considerations could lead to major contributions to conformational stability.

A test of this hypothesis on model compounds has been reported.[5] It has been demonstrated that the relative stability of the α-helices of several synthetic polypeptides, in non-aqueous media toward strong hydrogen bond breakers, such as dichloro and trichloroacetic acid, increases as the side-chain becomes less polar and more hydrocarbon in nature.

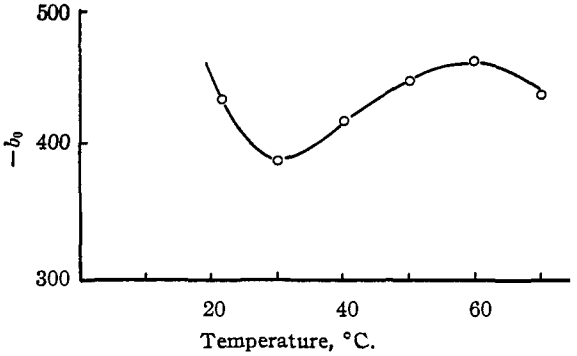

Fig. 2.—The b_0 values (from the Moffitt equation, λ_0 = 212 mμ, for the wave length range 365 to 589 mμ) as a function of temperature for copoly-L-glutamic acid:L-leucine, 65:35 (c = 0.25%; pH 5.18, 0.2 M NaCl).

We wish to report here a study in aqueous media directed toward examining the forces responsible for conformational stability. We have observed an increase in the stability of the helical conformation of copolypeptides of L-glutamic acid and L-leucine as compared to poly-L-glutamic acid (PGA). We have also found an inverse temperature effect wherein the helical content of several of these copolymers of different compositions first decreased with increasing temperature, and then upon a further increase of temperature, the helical content rose.

Copolymers of L-glutamic acid:L-leucine in mole ratios of 80:20 (I), 75:25 (II), and 65:35 (III), MW_w 55,000–70,000[11] were synthesized by the polymerization of γ-benzyl-L-glutamate-N-carboxyanhydride and L-leucine-N-carboxyanhydride (methoxide initiated) and subsequent removal of the benzyl groups *via* the HBr-HCl procedure.[12] The estimation of the relative stabilities, under identical aqueous conditions, of the helical conformation of these copolymers as compared to PGA, MW_w = 85,000 as a function of temperature was made by determining the b_0 values obtained from the Moffitt equation,[13] by Yang–Doty plots.[14] The b_0 value for a 100% right-handed helix has been found to be -630[13,14] (λ_0 = 212 mμ), while the random coil form has a value of zero.[14] (The b_0 plots represent the mean values of three independent determinations. The accuracy of the b_0 values is estimated to be ±15.)

The temperature–b_0 plot (Fig. 1) shows that the b_0 value of PGA at pH 4.88, 0.2 M NaCl, becomes less negative with increasing temperatures. The two copolymers, I and II, under the same conditions, at first display a similar change in b_0 values, becoming less negative, with increasing temperature, but at higher temperature an inversion occurs with b_0 becoming more negative, indicating an increase in helical content. In Fig. 2 is seen a

(1) W. Kauzmann, in "Advances in Protein Chemistry," Vol. XIV, C. B. Anfinsen, Jr., M. L. Anson, K. Bailey and J. T. Edsall, Eds., Academic Press, New York, N. Y., 1959, p. 1.

(2) H. A. Scheraga, *J. Phys. Chem.*, **65**, 1071 (1961); H. A. Scheraga, *Brookhaven Symposium in Biology*, **13**, 45 (1960).

(3) I. Klotz, *ibid.*, **13**, 25 (1960).

(4) H. A. Scheraga, G. Nemethy and I. Steinberg, *J. Biol. Chem.*, **237**, 2506 (1962); G. Nemethy and H. A. Scheraga, *J. Chem. Phys.*, **36**, 3382, 3401 (1962). G. Nemethy and H. A. Scheraga, *J. Phys. Chem.* **66**, 1773 (1962).

(5) G. Fasman, in "Polyamino Acids, Polypeptides and Proteins," M. Stahmann, Ed., University of Wisconsin Press, Madison, 1962, p. 221.

(6) C. Tanford, P. K. De, and V. G. Taggart, *J. Am. Chem. Soc.*, **82**, 6028 (1960).

(7) C. Tanford and P. K. De, *J. Biol. Chem.*, **236**, 1711 (1961).

(8) J. G. Foss, *Biochim. Biophys. Acta*, **47**, 569 (1961).

(9) D. T. Warner, *Nature*, **190**, 120 (1961).

(10) S. Lowey and C. Cohen, *J. Mol. Biol.*, **4**, 293 (1962).

(11) Intrinsic viscosities in the range of 1.68-1.50 at pH 6.8 at 0.2 M NaCl. Estimated weight average molecular weights.

(12) G. Fasman, M. Idelson and E. R. Blout, *J. Am. Chem. Soc.*, **83**, 709 (1961).

(13) W. Moffitt and J. T. Yang, *Proc. Nat. Acad. Sci. U. S.*, **42**, 596 (1956).

(14) J. T. Yang and P. Doty, *J. Am. Chem. Soc.*, **79**, 761 (1957).

similar plot for III studied at pH 5.18, 0.2 *M* NaCl. The b_0 value for PGA at 20° under similar conditions is −200. Thus by the inclusion of greater amounts of L-leucine in the polypeptide backbone of PGA the percentage helix increases at 20° under identical conditions. The observed temperature inversion and increase in helical content begins at lower temperature as the leucine content is increased.

These findings can best be understood by examining the temperature dependence of several interactions assumed responsible for helix stability such as hydrogen bonding, electrostatic interactions, and hydrophobic bonding. The contribution of hydrogen bonding to helical stability in aqueous solution decreases with increasing temperature[15-17] for the peptide hydrogen bonded backbone. It is plausible that a similar temperature effect is operative for hydrogen bonded pairs of un-ionized carboxyls[18] on the side chains, if such interactions play a significant role in conformational stabilization. The electrostatic repulsion between ionized carboxyls causing destabilization of the helix may decrease slightly due to a decrease in ionization upon heating.[18] However, this effect is not apparent in PGA alone. The effect of dilution of the charges in the copolymers as compared to PGA could possibly be responsible for their greater stability. While this is consistent with the greater stability of the copolymers toward heating, it is difficult to account for the increase in helical content at higher temperatures in terms of charge dilution. Thus it seems unlikely that these two forces, hydrogen bonding and electrostatic interactions could be responsible for the behavior of the copolymers in this study.

A third type of interaction, hydrophobic bonding, would be expected to be more important at elevated temperatures. These non-electrostatic side-chain interactions have been shown by Kauzmann[1] and others[2,4] to have a ΔH that is endothermic for the transfer of an aliphatic side-chain from water to a non-polar medium. In the random regions at room temperature the aliphatic side-chains are solvated by water and when the polypeptide becomes helical, this allows for more interactions among side-chains due to their juxtaposition along the helix, the latter occurring at elevated temperatures. In evaluating the role of the forces discussed above to account for the behavior of the copolymers it seems very probable that hydrophobic forces play the most important role.

Similar thermal inversions have been observed previously in non-aqueous solutions[19] and with some proteins in urea solutions.[15,16,20] However, the thermodynamic explanations offered always depend upon the mixed solvent systems used or specific binding which cannot be applied to the present study. A stable helix in aqueous media has been reported for a central block of poly-L-alanine in a block copolypeptide polymer,[21] but it was pointed out that there cannot be side-chain interactions in this case. More recently water soluble derivatives of copolymers of glutamic acid with methionine and alanine have been prepared which maintain their helical structure in aqueous solution.[22]

Acknowledgment.—This work was supported by a grant from the National Institutes of Health (A-5852).

(15) W. F. Harrington and J. A. Schellman, *Compt. rend. trav. lab. Carlsberg, Ser. Chim.*, **30**, 21 (1956).

(16) J. G. Foss and J. A. Schellman, *J. Phys. Chem.*, **63**, 2007 (1959).

(17) J. A. Schellman, *Compt. rend. trav. lab. Carlsberg, Ser. Chim.*, **29**, 230 (1955).

(18) P. Doty, A. Wada, J. T. Yang and E. R. Blout, *J. Polymer Sci.*, XXIII, 851 (1957).

(19) P. Doty and J. T. Yang, *J. Am. Chem. Soc.*, **78**, 498 (1956).

(20) F. G. Hopkins, *Nature*, **126**, 383 (1930).

(21) P. Doty and W. D. Gratzer, in "Polyamino Acids, Polypeptides, and Proteins," M. A. Stahmann, Ed., University of Wisconsin Press, Madison, Wis., 1962, p. 111.

(22) R. K. Kulkarni and E. R. Blout, *J. Am. Chem. Soc.*, **84**, 3971 (1962).

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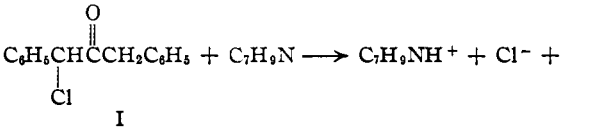
RECEIVED JULY 25, 1962

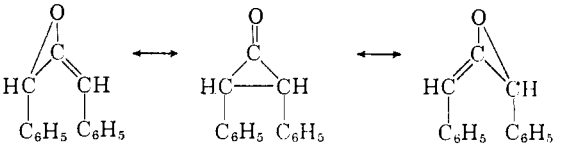
CAPTURE OF A FAVORSKII INTERMEDIATE BY FURAN

Sir:

An elimination–addition mechanism was proposed recently for the methanolysis of α-chlorodibenzyl ketone (I) promoted by 2,6-lutidine.[1] The kinetics of this reaction suggested that a proton and a chloride ion were lost from I to produce a reactive intermediate. Other lines of evidence

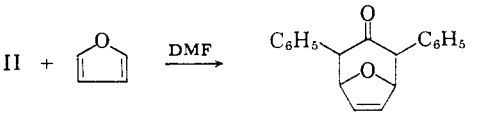


I



II

were advanced in support of the belief that the intermediate was a delocalized molecule, for which hybrid structure II was proposed, and it was suggested that the Favorskii rearrangement of I[2] proceeds through the same intermediate. New evidence that the reaction of I with 2,6-lutidine produces a delocalized molecule is now reported: when furan is present in the reaction mixture an adduct can be obtained.



III

In a small-scale experiment α-chlorodibenzyl ketone (I), 2,6-lutidine and furan, in a 1:4:2.5

(1) A. W. Fort, *J. Am. Chem. Soc.*, **84**, 2620 (1962).

(2) J. Jullien and P. Fauche, *Bull. soc. chim. France*, [5] **20**, 374 (1953).