fluoroacetic acid was found to have a "cross-β" structure[2] (see Fig. 1a). Stronger rubbing in one direction produced a film with a "parallel-β" structure (see Fig. 1b).

For the (a) film, the parallel dichroism of the 1632 cm.$^{-1}$ (amide I of a "β" conformation) and the 3328 cm.$^{-1}$ (NH stretching) bands, and the perpendicular dichroism of the 1514 cm.$^{-1}$ (amide II) band indicate that this film exists in a "cross-β" structure.[3] Furthermore, the perpendicular dichroism of the 1700 cm.$^{-1}$ band indicates antiparallel peptide alignment.[4,5] In the (b) film, the dichroism is weak compared with the (a) film, but suggests that this conformation corresponds to a "parallel-β" structure.

The molecular weight of this poly-O-acetyl-L-serine (Sample No. 211, which was synthesized by polymerization of O-acetyl-N-carboxy-L-serine anhydride) was estimated from the intrinsic viscosity value ($[\eta] = 0.12_3$) in trifluoroacetic acid, using the empirical equation[6] of Doty, Bradbury and Holtzer. It was *ca.* 10,000.

In connection with these observations two interesting points can be raised. First, some natural proteins, for example epidermin,[7] exhibit the "cross-β" to "parallel-β" transformation observed by us on poly-O-acetyl-L-serine. In addition, these proteins are rich in serine. It is supposed then, that the mechanism of "cross-β" and "parallel-β" transition is identical in natural proteins and poly-O-acetyl-L-serine.

Secondly, the synthetic polypeptides which have been reported with a pure "cross-β" structure are only of low degree of polymerization,[3,5] and in these cases the low molecular weight polypeptide chains seem to be running side by side at right angles to the fiber axis. In a polypeptide of not too low molecular weight, poly-β-benzyl-L-aspartate, the "ω" form and "cross-β" mixture obtained by heating to about 160° has been reported.[3] In the case of poly-O-acetyl-L-serine the molecular weight is not so low, while the "cross-β" structure is quite predominant. Therefore, two interpretations can be proposed for the structure of the "cross-β" configuration of poly-O-acetyl-L-serine: namely, the polypeptide chains are fully extended and align at right angles to the fiber axis as reported for low molecular weight "cross-β" structure, or one polypeptide chain is folded transversely like Rudall's model for epidermin.

The former requires interchain hydrogen bonds between peptide groups, while the latter would have intrachain hydrogen bonds. There has been no direct confirmation of either bond type in the structure of poly-O-acetyl-L-serine. However, if the "cross-β" structure observed in the solid state exists in solution where "β" conformation has been assigned from infrared solution spectroscopy,[1,8] indirect evidence for the existence of the intrachain "cross-β" structure is found by its behavior on dilution. The values of the optical rotation of poly-O-acetyl-L-serine at $\lambda = 546$ mμ in solutions of dichloroacetic acid or trifluoroacetic acid (10–25%) and chloroform (90–75%) mixtures are little dependent on the polymer concentration[8] while the $[\alpha]_{546}$ of interchain "β" polypeptide is remarkably dependent on the concentration.[9,10] These observations on the concentration dependence of optical rotation suggest that the intramolecular "β" structure exists in poly-O-acetyl-L-serine solution. As a further evidence, detailed studies on X-ray analysis have been carried out[11] though some preliminary X-ray works have been suggested to be done.[2]

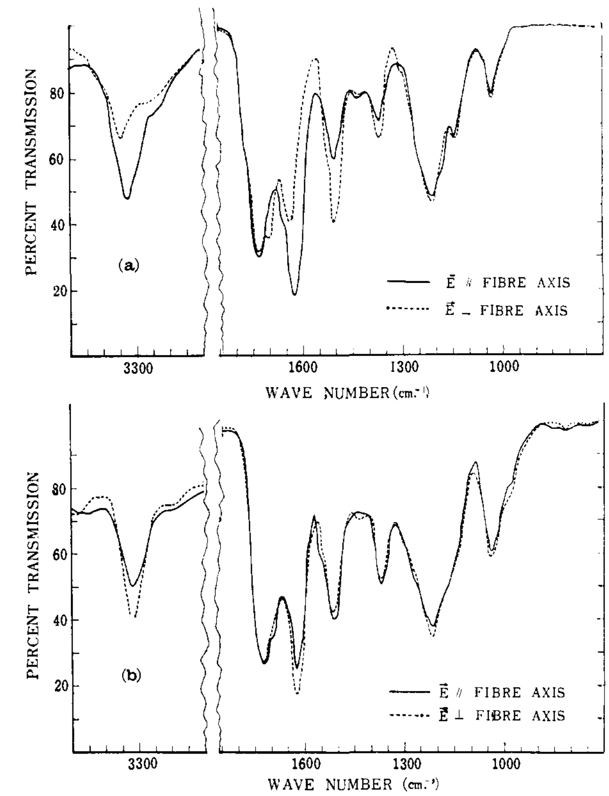

Fig. 1.—Infrared spectra of oriented film of poly-O-acetyl-L-serine: ——, electric vibration direction parallel to fiber axis; – – –, electric vibration direction perpendicular to fiber axis; (a) shows cross-β structure; (b) shows parallel-β structure.

(2) C. Johnson and C. Cohen "quoted in reference 1" assigned the cross-β structure to poly-O-acetyl-L-serine from X-ray diffraction investigation.

(3) E. M. Bradbury, L. Brown, A. R. Downie, A. Elliott, D. B. Fraser, W. E. Hanby and T. R. R. McDonald, *J. Mol. Biol.*, **2**, 276 (1960).

(4) T. Miyazawa and E. R. Blout, *J. Am. Chem. Soc.*, **83**, 712 (1961).

(5) M. Tsuboi and A. Wade, *J. Mol. Biol.* **3**, 480 (1961).

(6) P. Doty, J. A. Bradbury and A. M. Holtzer, *J. Am. Chem. Soc.*, **78**, 947 (1956).

(7) K. M. Rudall, *Advances in Protein Chem.*, **7**, 253 (1952).

(8) I. Yahara and K. Imahori, in preparation.

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

(10) A. Wada, M. Tsuboi and E. Konishi, *J. Phys. Chem.*, **65**, 1119 (1961).

(11) I. Yahara, K. Imahori, Y. Iitaka and M. Tsuboi, *J. Polym. Sci.*, in press.

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THE DETERMINATION OF THE STABILITIES OF SCHIFF BASE COMPLEXES INVOLVING DISSOCIATED SCHIFF BASES

Sir:

Increasing interest has lately been focused on the properties of Schiff base complexes especially with regard to their role in non-enzymatic transamination reactions.[1–7] While the stabilities of the complexes with the more stable Schiff bases can be obtained readily[8] few studies seem to have been reported concerning those complexes in which the Schiff base itself is appreciably dissociated. The investigations described in refs. 2, 4, 5, and 7 concern such cases. These investigations were carried out spectrophotometrically and because of the difficulties encountered in interpreting the results when spectrally similar species are involved it was necessary either to arrange conditions so only Schiff base complexes having a ratio of metal ion

(1) D. E. Metzler and E. E. Snell, *J. Am. Chem. Soc.*, **74**, 979 (1952).

(2) G. L. Eichhorn and J. W. Dawes, *ibid.*, **76**, 5663 (1954).

(3) B. Witkop and T. W. Beiler, *ibid.*, **76**, 5589 (1954).

(4) H. N. Christensen, *ibid.*, **80**, 2305 (1958).

(5) L. Davis, F. Roddy and D. E. Metzler, *ibid.*, **83**, 127 (1961).

(6) H. Mix, *Z. physiol. Chem.* **315**, 1 (1959).

(7) L. J. Nunez and G. L. Eichhorn, *J. Am. Chem. Soc.*, **84**, 901 (1962).

(8) T. J. Lane and A. J. Kandathil, *ibid.*, **83**, 3782 (1961).

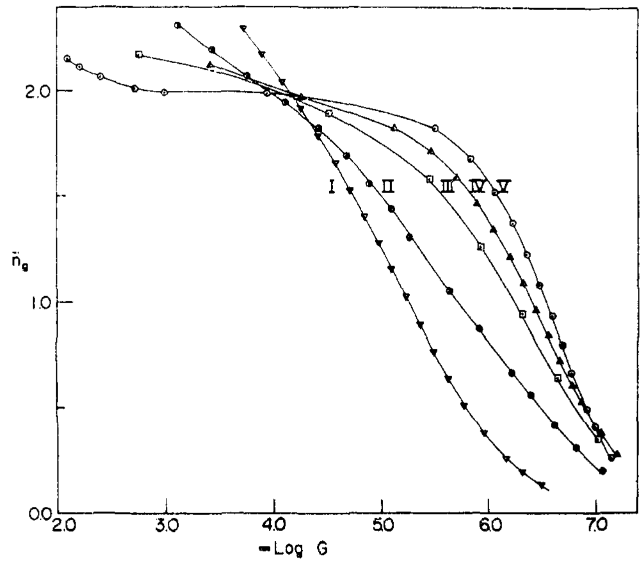


Fig. 1.—The effect of pyruvate on the complexing of glycinate by nickel(II): pyruvate concentration, I, 0.00 *M;* II, 0.05 *M;* III, 0.10 *M;* IV, 0.15 *M;* V, 0.15 *M*. The Ni(II) concentration is 0.050 *M* in all cases except for V where it is 0.020 *M;* 0.50 *M* KCl, 25°.

to ligand of 1:1 predominated or it was assumed that only such a Schiff base complex was present. Evidence of higher species however has been obtained for solution[3] and solid[5] phases.

It is valuable to consider the Schiff base complexes as "mixed" complexes since it does not matter in the mathematical treatment of the data, whether the carbonyl compound and the amine are independently coördinated to the metal ion or are combined as a Schiff base. Watters[9] has demonstrated that the pH method developed by Bjerrum[10] can be applied successfully to systems of "mixed" complexes when the two different ligands have sufficiently varying acid–base properties. In these laboratories we are currently studying Schiff base complexes and we wish to report the successful application of Watters' concept to pyruvate–glycinate "mixed" complexes.

The acid dissociation constant of pyruvic acid is 4.07×10^{-3} (0.50 *M* KCl, 25°) and for glycine K_{1a} and K_{2a} are 3.43×10^{-3} and 2.01×10^{-10}. Therefore, in solutions containing known total concentrations of a complexing metal ion, pyruvic acid and sodium glycinate and in which the equilibrium pH values are 4.3 or higher, the amount of complexed glycinate ion can be calculated using the results of pH measurements. For Ni(II) systems the experimental values of $\bar{n}_G$ (G_{comp}/Ni_t) are shown in Fig. 1.

For divalent metal ions of the first transition series the mass relationships are described by the equations

$$M_t = (M^{++}) + (MG^+) + (MG_2) + (MG_3^-) + (MP^+) + (MPG) + (MPG_2^-) + (MP_2G_2^=)$$

$$P_t = (P^-) + (MP^+) + (MPG) + (MPG_2^-) + 2(MP_2G_2^=) + (PG^=)$$

$$G_{comp} = (MG^+) + 2(MG_2) + 3(MG_3^-) + (MPG) + 2(MPG_2^-) + 2(MP_2G_2) + (PG^=)$$

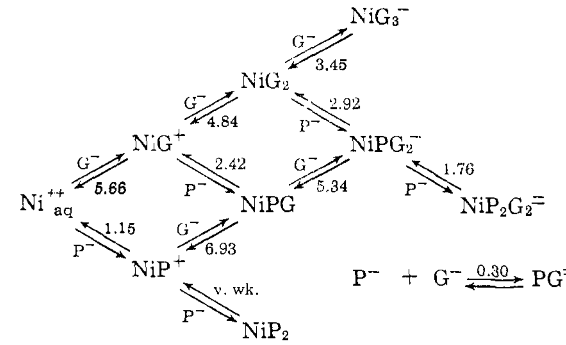
where the quantities on the right-hand side represent the known total concentrations of M(II), pyruvate and glycinate and those on the left hand side represent the equilibrium concentrations of the various species. Terms in the concentration of MP_2 have been omitted

(9) (a) J. I. Watters, *J. Am. Chem. Soc.*, **81**, 1560 (1959); (b) J. I. Watters and R. De Witt, *ibid.*, **82**, 1333 (1960).

(10) J. Bjerrum "Metal Ammine Formation in Aqueous Solution," P. Haase and Son, Copenhagen, 1941.

(and accordingly those in MP_2G) because of the low stability of these species.

The step-wise equilibria and the logarithms of the stepwise constants which have been found to describe the Ni(II) system are[11]



These results show that each of the species NiPG, $NiPG_2$ and $NiP_2G_2^=$ has an appreciable stability and none can be safely ignored. It is interesting to note how the previous coordination of one of the moieties of the "mixed" species enhances the coördination of the other in spite of statistical and electrical effects which have been observed[9b] in mixed complexes to operate in the other direction. This large "rest effect"[10] suggests that Schiff base formation occurs in the "mixed" complexes. It is also interesting to note the relatively large value of the constant for the step $NiG_2 + P^- \rightarrow NiP_2^-$ compared to the analogous steps $NiG^+ + P^- \rightarrow NiGP$ and $NiPG_2^- + P^- \rightarrow NiP_2G_2^=$. While any conclusions at the present time are tentative, this result may indicate that the rate of establishment of the equilibrium

$$Ni(G')(P{=}G)^- \rightleftharpoons Ni(G'{=}P)/(G^-)$$

is rapid compared to the over-all formation or dissociation rates of the complex. Equilibration periods of the order of 5-30 minutes were observed in this study.

Transamination was also found to occur in the Ni(II)-pyruvate–glycinate system. Solutions after the last addition of sodium glycinate (pH $\sim$ 9) were allowed to stand at room temperature for about 60 hours. After this time the complex was decomposed by adding EDTA which removed the stabilizing Ni(II) ions. Paper electrophoresis showed that appreciable amounts of alanine had been formed.

Ca(II), Mn(II) and Zn(II) ions also have been found to form complexes having the composition MPG and $MP_2G_2^=$ but these ions do not appear to form MPG_2^-.

These studies are being continued and will be reported in greater detail in future publications.

The author wishes to express his appreciation to the American Dental Association for its financial support of this work in its initial phase at the National Bureau of Standards.

(11) Solutions were obtained using a high speed digital computer. Programs were written using the OMNITAB routine developed at the National Bureau of Standards by J. Hilsenrath and G. Ziegler.

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RECEIVED OCTOBER 27, 1962

OXOFLUORINATION—A NEW BIDENT REACTION OF PERCHLORYL FLUORIDE

Sir:

Perchloryl fluoride (PF) is a stable, tetrahedrally symmetrical molecule, which reacts as an ambident electrophile.[1] No isolated, or conjugated, carbon–

(1) A. S. Kende and P. MacGregor, *J. Am. Chem. Soc.*, **83**, 4197 (1961).