cine,[2] *cis*- and *trans*-3-hydroxyproline,[3] serine, *allo*-threonine, and threonine. Basic hydrolysis produces tryptophan and β-methyltryptophan.[4] Quantitative amino acid determinations by both the Moore–Stein technique and mass spectrometry show that only one of each amino acid is present. None of the amino acids was attacked by D-amino acid oxidase. Potentiometric titration indicated that telomycin has one free carboxyl function and one free amino group; molecular weight determinations averaged 1300. The molecular weight of Telomycin is calculated at 1273.3 on the basis of the eleven amino acids linked by ten peptide bonds and one lactone. The presence of a lactone is confirmed by the disappearance of the characteristic ester-type infrared band at 1745 cm.$^{-1}$, accompanied by a change in optical rotation [from $[\alpha]^{28}D$ −133° to $[\alpha]^{28}D$ −32°; (*c* 1, 2:1 methanol–water)] on treatment with 0.32 *N* barium hydroxide at ambient temperature. Potentiometric titration of the newly formed "telomycic acid" disclosed an additional carboxylic acid function ($pK_a$ 3.6). Chromic acid oxidation[5] showed that threonine is protected in Telomycin but not in telomycic acid, thus establishing threonine as the O-terminus of the lactone. As expected, Telomycin travels to the cathode while telomycic acid migrates to the anode on electrophoresis at pH 6.4.

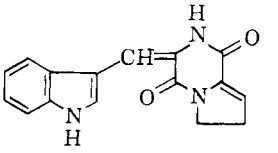
Although attempts to degrade Telomycin or telomycic acid to peptides by means of acidic or enzymatic partial hydrolysis were unpromising, partial basic hydrolysis was successful. Prolonged treatment with aqueous sodium hydroxide, followed by ion-exchange neutralization, gave (in addition to traces of amino acids) five water-soluble peptides and one water-insoluble hexapeptide.

The water-soluble peptides were separated by electrophoresis, yielding a pentapeptide (Asp, Ser, Thr, *allo*-Thr, Ala); a tetrapeptide (Asp, Ser, Threo, *allo*-Thr); two tripeptides (Asp, Ser, Thr) and (Thr, *allo*-Thr, Ala); and a dipeptide (Asp, Ser). The pentapeptide had aspartic acid as the N-terminal amino acid, as did Telomycin itself (Sanger DNFB method). Hydrazinolysis (Akabori) of the pentapeptide revealed alanine as C-terminal. These observations, together with p*K* studies, establish the N-terminal sequence of telomycin as β-Asp-Ser-Thr-*allo*-Thr-Ala.

The purified (thin-layer chromatography) water-insoluble hexapeptide contains both 3-hydroxyprolines, glycine, tryptophan, β-methyltryptophan, and β-hydroxyleucine; the N-terminal amino acid is glycine (Sanger DNFB). Since the C-terminal amino acid of telomycic acid is *cis*-C-hydroxyproline (Akabori), this must also be the position occupied in the hexapeptide.

The Edman technique confirmed glycine as N-terminal and revealed *trans*-3-hydroxyproline as the next amino acid in the hexapeptide. Partial hydrolysis (acidic) of the hexapeptide gave a tripeptide containing both tryptophans and β-hydroxyleucine (acidic and basic total hydrolyses). Edman degradation on this tripeptide showed β-hydroxyleucine to be N-terminal and hydrazinolysis produced only tryptophan.

Alkaline hydrolysis under nitrogen of either the hexapeptide or Telomycin gave indole-3-aldehyde and a yellow crystalline product, m.p. 280–283° dec. *Anal.* Calcd. for $C_{16}H_{13}O_2N_3$: C, 68.80; H, 4.69; N, 15.05; mol. wt., 279.29. Found: C, 68.71; H, 4.81; N, 15.02; mol. wt., 279.1 (mass number). The ultraviolet spectrum shows a band at 380 mμ (ε 16,000). Hydrogenation over rhodium–charcoal followed by acid hydrolysis gave proline and hydrotryptophan. These data are in good accord with the formulation of the yellow, crystalline product I.

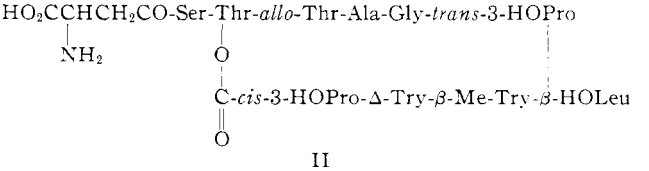


I

The ultraviolet spectra of Telomycin, telomycic acid, and the hexapeptide all have, in addition to tryptophan-type absorption, a band at 339 mμ (ε 22,000).

This ultraviolet chromophore can be accounted for by a dehydrotryptophan system. For example, methyl β-(3-indolyl)-α-benzamidoacrylate [prepared by the Erlenmeyer condensation of β-indolealdehyde and hippuric acid followed by methanol] has an absorption band at 341 mμ (ε 21,000).

All of the foregoing observations are compatible with the representation of Telomycin as structure II.



II

The formation of tryptophan from the dehydrotryptophan system on alkaline hydrolysis is surprising, and this type of reaction is being investigated further.

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(2) J. C. Sheehan, K. Maeda, A. K. Sen, and J. A. Stock, *J. Am. Chem. Soc.*, **84**, 1303 (1962).

(3) The *trans* configuration has been assigned to the "slow-moving" 3-hydroxyproline on the basis of the classically rigorous conversion of its precursor, 3-methoxy-L-proline, to L-methoxysuccinamide. J. C. Sheehan and J. G. Whitney, *ibid.*, in press.

(4) By electrophoresis and paper chromatography the β-methyltryptophan from Telomycin has been shown to correspond to synthetic "A" racemate kindly provided by Professor H. R. Snyder of the University of Illinois. *Cf.* H. R. Snyder and D. S. Matteson, *J. Am. Chem. Soc.*, **79**, 2217 (1957). The β-methyltryptophan structure was suggested by Professor Klaus Biemann (M.I.T.) on the basis of mass spectrometric measurements on the isolated amino acid furnished by our laboratory. *Cf.* K. Biemann, "Mass Spectrometry," McGraw-Hill Book Co. Inc., New York, N. Y., 1962, p. 275.

(5) J. C. Sheehan, H. G. Zachau, and W. B. Lawson, *J. Am. Chem. Soc.*, **80**, 3349 (1958).

## The Separation of Ketimine Isomers

*Sir:*

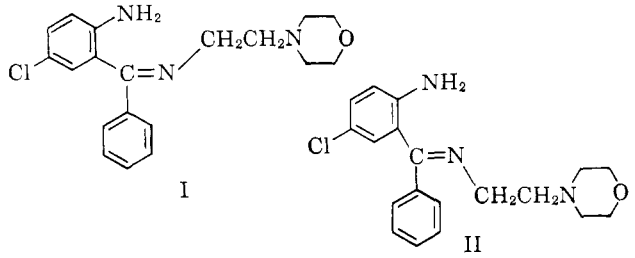
Earlier claims of the separation of the geometric isomers of aldimines and ketimines[1] have been disputed.[2] We have now succeeded in preparing and separating both forms of a number of ketimines derived from 2-amino-5-chlorobenzophenone.

These compounds are typified by the morpholinoethylimines, obtained by heating 2-amino-5-chloro-

(1) O. Anselmino, *Ber.*, **40**, 3465 (1907); W. Manchot and J. R. Furlong, *ibid.*, **42**, 3030 (1909); M. E. Taylor and T. L. Fletcher, *J. Am. Chem. Soc.*, **80**, 2246 (1958).

(2) V. De Gaouck and R. J. W. Le Fèvre, *J. Chem. Soc.*, 741 (1938); *ibid.*, 1392 (1939); D. Y. Curtin and J. W. Hausser, *J. Am. Chem. Soc.*, **83**, 3474 (1961).

benzophenone and aminoethylmorpholine in xylene under reflux in the presence of zinc chloride. Following solvent removal, fractional crystallization of the residue from alcohol and hexane gave a less soluble $\alpha$-form I, m.p. 140–142° (*Anal.* Calcd. for $C_{19}H_{22}ClN_3O$: C, 66.36; H, 6.45; N, 12.22. Found: C, 66.13; H, 6.29; N, 12.09.) and a more soluble $\beta$-form II, m.p. 112–114° (Found: C, 66.17; H, 6.50; N, 12.43). Both I and II upon treatment with aqueous acid returned the starting materials. Heating II without a solvent at 140–150° for 10 min. afforded I.




The infrared and n.m.r. spectra of I and II were consistent with the imine formulations.

Compounds I and II had different ultraviolet absorption spectra: I, $\lambda_{max}^{EtOH}$ 248 m$\mu$ ($\epsilon$ 25,300); II, $\lambda_{max}^{EtOH}$ 233 ($\epsilon$ 27,100), 362 m$\mu$ ($\epsilon$ 4,840). The $\alpha$- and $\beta$-oximes of 2-amino-5-chlorobenzophenone (III and IV) whose configurations have been firmly established[3] as *syn* and *anti*, respectively, to the substituted phenyl had the following ultraviolet absorption spectra: III, $\lambda_{max}^{EtOH}$ 246 m$\mu$ ($\epsilon$ 21,700); IV, $\lambda_{max}^{EtOH}$ 231 (26,000); 346 m$\mu$ ($\epsilon$ 8,500). Compound I, therefore, has a *syn* configuration to the substituted phenyl with II having an *anti* arrangement. The absorption bands above 320 m$\mu$ in II and IV are believed to be due to the chromophores arising from conjugation of the *o*-aminophenyl group with the imino bond which is possible only when the nitrogen substituent is *anti*. Steric crowding does not permit the rings to be coplanar.[4]

The assigned configurations are supported by the absorption spectrum of N-(2-amino-5-chloro-$\alpha$-phenylbenzylidene) glycine, sodium salt, $\lambda_{max}^{H_2O}$ 240 m$\mu$ ($\epsilon$ 17,500), which was obtained[5] by opening 7-chloro-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one with alkali and which must have its nitrogen substituent *syn* to the amino substituted phenyl group.

Further, the absorption spectrum of benzophenone morpholinoethylimine, b.p. 180–183° (0.3 mm.) (*Anal.* Calcd. for $C_{19}H_{22}N_2O$: C, 77.51; H, 7.53; N, 9.52. Found: C, 77.74; H, 7.54; N, 9.23.), in which the imino bond can only be in conjugation with an unsubstituted phenyl group, exhibited no maximum above 300 m$\mu$. This is true also for benzophenone oxime.

(3) L. H. Sternbach, S. Kaiser, and E. Reeder, *J. Am. Chem. Soc.*, **82**, 475 (1960); T. S. Sulkowski and S. J. Childress, *J. Org. Chem.*, **25**, 4424 (1962).

(4) P. A. S. Smith and E. P. Antoniades, *Tetrahedron*, **9**, 210 (1960).

(5) S. C. Bell, T. S. Sulkowski, C. Gochman, and S. J Childress, *J. Org. Chem.*, **25**, 562 (1962).

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## Comparison of Hypochromic Effect and Optical Rotation in Poly-L-glutamic Acid

*Sir:*

We wish to report some data which resolve a controversy over the relationship between the helical conformation of polypeptides and the hypochromic effect in the amide absorption band at 190 m$\mu$ discovered by Imahori and Tanaka.[1] The idea that the decrease in the extinction coefficient at this wave length could be simply correlated with the extent of helix formation was called into question by Wada,[2] Applequist,[3] and Miyazawa[4] on the grounds that the extinction coefficient for poly-L-glutamic acid (PGA) was not simply correlated with the optical rotation and in fact seemed to follow more nearly the degree of ionization of the carboxyl groups. It was recognized, however, that the various experiments being compared were carried out under different conditions, particularly regarding concentration of polypeptide, and that the results of the comparison were therefore inconclusive. In order to obtain more decisive information, we have carried out optical rotation, ultraviolet absorption, and titration measurements on identical solutions of PGA.

PGA (Pilot Chemicals Lot G-13, intrinsic viscosity = 1.56 dl./g. in 0.2 *M* NaCl, pH 7) was dissolved with the aid of dilute NaOH to a concentration of 0.50 g./dl. (based on carboxyl form) in the presence of 0.2 *M* NaCl. Titration of the solution with 0.8 *M* HCl was followed with a Beckman Model G pH meter. The volume change due to addition of acid from pH 7 to 4 was less than 4% and was not corrected for in the concentration. Optical rotations at 589 m$\mu$ were measured with a Rudolph spectropolarimeter in a 20-cm. polarimeter tube, giving measured rotations from $-0.1°$ to $-1°$ with a precision of $\pm 0.01°$. Absorption spectra were measured on a Cary Model 14 spectrophotometer in a cell of 0.01 cm. path length (Quaracell Products, Inc.). At 190 m$\mu$ absorbances were in the range of 2 to 3. Large errors at this wave length due to noise and chloride absorption (arising from slight mismatching of sample and solvent cells) precluded accurate work, and we chose instead to make measurements on the side of the absorption band, where the hypochromic effect is still easily observable. The wave length 200 m$\mu$ seemed optimum for this purpose, as the absorbances were in the range of 1 to 2 and the precision $\pm 0.005$. Extinction coefficients showed less precision, however, due to variations in path length which occur when the cell is dismantled for refilling. Rotation and extinction coefficient measurements were therefore of comparable precision.

The results are shown in Fig. 1. It is seen that the change in extinction coefficient follows rather closely the change in rotation, and that neither of these is a linear function of the degree of ionization. It, therefore, appears that the discrepancies noted earlier can be attributed to differences in conditions of measurement. Furthermore, it is now evident that Imahori and Tanaka were correct in attributing the hypochromic effect to interactions in the helical conformation, since the change in rotation has been shown[5] to result from helix formation at low pH. This conclusion is of particular interest in view of the success of Tinoco, *et al.*,[6] in predicting the magnitude of the hypochromic effect from dipole–dipole interactions in the helix. It does not follow from these observations that either rotation or absorption is *linearly* related to the helix content, but merely that both are similar functions of helix content. The linear relationship generally assumed is neither confirmed nor contradicted.

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

(2) A. Wada, in "Polyamino Acids, Polypeptides, and Proteins," M. A. Stahmann, Ed., University of Wisconsin Press, Madison, Wisconsin, 1962, p. 157.

(3) J. Applequist, *ibid.*, p. 158.

(4) T. Miyazawa, *ibid.*, p. 159.

(5) P. Doty, A. Wada, J. T. Yang, and E. R. Blout, *J. Polymer Sci.*, **23**, 851 (1957).

(6) I. Tinoco, Jr., A. Halpern, and W. T. Simpson, "Polyamino Acids, Polypeptides, and Proteins," M. A. Stahmann, Ed., University of Wisconsin Press, Madison, Wisconsin, 1962.