weight compounds may be related to the effects observed when very thin liquid films are employed[2]; for example, cholesterol required only about 35 min. for elution at 0.68 atm. inlet pressure (30 ml./min. flow rate) with a 183 cm. column at 222°.

Stereochemical change in the A/B ring junction led to different retention times for otherwise similar compounds. Hydroxy compounds were eluted before the corresponding ketones, without trailing; ketones showed some trailing. A considerable difference in retention time was observed with increasing carbon content. Both columns had about 2300 theoretical plates (cholestane).

The usefulness of this phase for other kinds of high molecular weight compounds has not been investigated. Silicone grease phases are known to have high thermal stability,[3,4] and while the present phase may be used to 250–300° its greatest value may lie in its unusual ability to resolve steroids rapidly at relatively low temperatures.[5]

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[2] C. Hishta, J. P. Messerly and R. F. Reschke, Abstracts 137th A.C.S. Meeting, April, 1960, p. 29-B.

[3] J. Cason and W. T. Miller, *J. Org. Chem.*, **24**, 1814 (1959).

[4] C. Asselineau, J. Asselineau, R. Ryhage, S. Stallberg-Stenhagen and E. Stenhagen, *Acta Chem. Scand.*, **13**, 822 (1959).

[5] Sterol analysis on a silicone column, operated at 287°, has been described recently (R. K. Beerthuis and J. H. Recourt, *Nature*, **186**, 372 (1960)).

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FAST REACTIONS OF IMIDAZOLE STUDIED WITH RELAXATION SPECTROMETRY

Sir:

The understanding of enzymatic reactions often is hindered because of the difficulty of isolating and studying individual steps in the mechanisms. In many enzymatic systems, it appears that imidazole is involved intimately in the catalytic process. For these reasons, studies of the kinetics of the fast reactions of imidazole with hydrogen ion, hydroxyl ion and a proton donor–acceptor system in the region of neutrality (chlor phenol red) were undertaken with relaxation techniques.

Two experimental approaches were employed: the dissociation field[1] and the temperature jump methods.[2] Both utilize the same principle: perturbation of a system at chemical equilibrium and determination of the relaxation time (or times) necessary for the reestablishment of equilibrium. The measured spectrum of relaxation times can be related to the rate constants of the chemical reactions in the system.[3] Detailed descriptions of the experimental apparatus and procedures can be found elsewhere.[1,2,4,5]

The dissociation field method was used to study the kinetics of the reaction

$$\underset{\text{I}}{\mathrm{ImH^+ + OH^-}} \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} \underset{\text{II}}{\mathrm{Im + H_2O}} \qquad (1)$$

in the neighborhood of pH 10. Here ImH$^+$ represents the imidazolium ion and Im the neutral imidazole molecule. The relaxation time, τ, in this case is

$$\tau = [k_{21} + k_{12}(C_{\mathrm{OH^-}} + C_{\mathrm{ImH^+}})]^{-1} \qquad (2)$$

Since the equilibrium constant is known,[6] both rate constants could be determined (at essentially zero ionic strength and 25°).

The system studied with the temperature jump consisted of imidazole, chlor phenol red (a pH indicator with pK 6.08, through which pH changes could be followed spectrophotometrically), and 0.1 M KNO$_3$. The measurements were carried out in the pH range of 5.5 to 6.5 at 13°. The reaction mechanism can be written as

$$\begin{tikzcd}
& \overset{\text{II}}{\mathrm{Im + H^+ + In^{-2}}} \arrow[dl, shift left] \arrow[dr, shift left, "24"] & \\
\underset{\text{III}}{\mathrm{ImH^+ + In^{-2}}} \arrow[ur, shift left, "23"] \arrow[rr, shift left, "34"] & & \underset{\text{IV}}{\mathrm{Im + HIn^-}} \arrow[ul, shift left, "42"] \arrow[ll, shift left, "43"]
\end{tikzcd}$$

The state II in this system differs from that in equation 1 by the presence of dissociated indicator. Furthermore, In^{-2} and HIn$^-$ represent the forms of the indicator important in the pH range under consideration. This mechanism is characterized by two relaxation times which are complicated (but known) functions of the six rate constants and the equilibrium concentrations. Only the longer relaxation time (>10 μsec.) could be measured, although a shorter relaxation time (<5 μsec.) could be detected. By varying concentrations and using a trial and error procedure to fit the data, all six rate constants were determined. The rate constants obtained are:

M^{-1} sec.$^{-1}$	sec.$^{-1}$	%
$k_{12} = 2.3 \times 10^{10}$	$k_{21} = 2.3 \times 10^{3}$	(±15)
$k_{23} = 1.5 \times 10^{10}$	$k_{32} = 1.5 \times 10^{3}$	(±35)
$k_{24} = 3 \times 10^{10}$	$k_{42} = 2.4 \times 10^{4}$	(±50)
$k_{43} = 9 \times 10^{8}$	$k_{34} = 1.2 \times 10^{8}\ M^{-1}$	(±35)

The relatively large experimental error in the rate constants determined by the temperature jump method is due to the evaluation from the complicated form of the relaxation time since individual relaxation times can be found to ±10%.

The rates of reaction of imidazolium ion and imidazole with hydroxyl and hydrogen ions, respectively (k_{12}, k_{23}), are diffusion controlled. It is interesting to note that at pH 7 and at salt concentrations usually present in physiological systems, the two rates would be about equal. The reported value of k_{12} is valid for zero ionic strength, whereas k_{23} is determined at an ionic strength of 0.1. The latter value, however, should be fairly independent of ionic strength since no net change in charge is involved. The rate of combination of chlor phenol red and a proton (k_{24}) is also diffusion controlled and is identical with the value previously found for a similar reaction of phenol red.[7] On the other hand, the rate of pro-

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[1] M. Eigen and J. Schoen, *Z. Elektrochem.*, **59**, 483 (1955).

[2] G. Czerlinski and M. Eigen, *ibid.*, **63**, 652 (1959).

[3] M. Eigen, *ibid.*, **64**, 115 (1960).

[4] L. De Maeyer, *ibid.*, **64**, 65 (1960).

[5] H. Diebler, Ph.D. Thesis, in preparation.

[6] A. H. M. Kirby and A. Neuberger, *Biochem. J.*, **32**, 1146 (1938).

[7] H. Diebler, M. Eigen and G. G. Hammes, *Z. Naturforsch.*, (b) in press (1960).

ton transfer between imidazole and chlor phenol red (k_{43}), appears to be slightly smaller than the value expected for a diffusion controlled process.[3] Weller[8] also found similar rates for reactions with acridine ($k \sim 5 \times 10^8\ M^{-1}$ sec.$^{-1}$) which suggests that this rate either is characteristic of proton transfers involving a nitrogen atom or depends somewhat on the pK-values of the proton donor–acceptor system. For such transfer processes, diffusion controlled rates ($k \sim 3 \times 10^9\ M^{-1}$ sec.$^{-1}$) have been found[8] only when the proton was transferred between two oxygen atoms which were more acidic ($pK \sim 5$) than the systems considered above.

The value of k_{12} should be compared to previous results obtained for similar reactions between a cation and hydroxyl ion; these are listed in Table I. All these rate constants are characteristic of diffusion controlled processes; the differences in rates are due to steric factors. Thus, ammonia is the fastest because it has four sites for proton transfer. Trimethylamine ion, on the other hand, is sterically hindered by the methyl groups and has a correspondingly slower rate of reaction. The fact that the imidazole rate constant is twice as large as that for trimethylamine indicates that the reaction is able to take place at both nitrogen atoms in the imidazolium ion ring. This occurrence is due either to resonance stabilization which makes the two nitrogens equivalent, or to a fast tautomerization of the ring after reaction at the neutral nitrogen. Acridine would be expected to be less sterically hindered than trimethylamine, but should react more slowly than imidazole, which has two reactive nitrogen atoms. This fact is in accord with the intermediate value of the rate constant that was found.

TABLE I

Cation	k_{12}(M^{-1} sec.$^{-1}$)	Reference
NH_4^+	3.0×10^{10}	1
Acridinium	1.85×10^{10}	8
$(CH_3)_3NH^+$	1.0×10^{10}	9

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(8) A. Weller, *Z. Elektrochem.*, **64**, 55 (1960).

(9) M. Eigen, J. S. Johnson, K. Kustin and A. Wittig, in prepn.

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CONFORMATIONAL ASPECTS OF SYNTHETIC POLYPEPTIDES. II. CRITICAL RANGE FOR INTRAMOLECULAR HYDROGEN BONDING

Sir:

In our previous report,[1] we employed optical activity to measure the conformation[2-9] of oligomeric compounds derived from γ-methyl-L-glutamic acid. The optical activities were determined in both random coil and helix-forming solvents. However, association complicated interpretation of the results.[1,10] Molecular weight determinations using short column equilibrium ultracentrifugation confirmed association.[11] In this communication we wish to report optical activity enhancements and abnormal rotatory dispersions for the oligomeric peptides in solvents where no association occurs. When the oligomeric peptides are studied in dimethylformamide and *m*-cresol, concentration independent optical activity enhancements are noted (Table II). The lack of association was confirmed by molecular weight determination in dimethylformamide.[11] Table I contains a list of the optical rotations and rotatory dispersion b_0 values from the Moffitt equation[12] for polymers of poly-γ-methyl-L-glutamates in dimethylformamide and dichloroacetic acid.

TABLE I

OPTICAL ROTATION DATA ON POLY-γ-METHYL-L-GLUTAMATES

Solvent	A/I	$[\alpha]^{25}_D$	b_0[a]
Dimethylformamide	High	+10	−544
Dimethylformamide	93	+10	−532
Dimethylformamide	22	+ 8	−486
Dichloroacetic acid	104	−33	+ 56

[a] Corrected for refractive index of solvent; a value of 212 mμ was used as λ_0.

Rotatory dispersion as a criterion for helix content has been established and examined by Moffitt,[12] Doty,[2] Cohen,[13] Tinoco,[14] Blout[15] and Elliott.[16] Moffitt expanded the Drude equation to include a higher order term.[12] He suggested that a plot of $[\alpha](\lambda^2-\lambda_0^2)$ *vs.* $(\lambda^2-\lambda^2_0)^{-1}$ with other than a zero slope exhibits abnormal rotatory dispersion, indicating helical structures. Doty[2] suggested the use of λ_c[17] as a criterion of helicity for some synthetic and naturally occurring polypeptides.

We determined the optical rotatory dispersion of the oligomeric compounds in helix and random coil solvents. In dichloroacetic acid Moffitt plots indicated material essentially of a random coil nature. In dimethylformamide, however, increasingly negative abnormal dispersions were observed in going from the trimer to the undecamer (Fig. 1) (Table II).

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(1) M. Goodman and E. E. Schmitt, THIS JOURNAL, **81**, 5507 (1959).

(2) J. T. Yang and P. Doty, *ibid.*, **79**, 761 (1957).

(3) P. Doty, A. M. Holtzer, J. H. Bradbury, E. R. Blout, *ibid.*, **76**, 4493 (1954).

(4) P. Doty and J. T. Yang, *ibid.*, **78**, 498 (1956).

(5) P. Doty and R. D. Lundberg, *Proc. Natl. Acad. Sci.*, **43**, 213 (1957).

(6) A. R. Downie, A. Elliott, W. E. Hanby and B. R. Malcolm, *Proc. Roy. Soc.* (*London*), **A242**, 325 (1957).

(7) I. Tinoco, THIS JOURNAL, **81**, 1541 (1959).

(8) C. Cohen, *Nature*, **175**, 129 (1955).

(9) E. R. Blout, P. Doty and J. T. Yang, THIS JOURNAL, **79**, 749 (1957).

(10) P. Doty, J. H. Bradbury and A. M. Holtzer, *ibid.*, **78**, 947 (1956).

(11) D. Yphantis, *Ann. N. Y. Acad. Sci.*, to be published.

(12) (a) W. Moffitt, *J. Chem. Phys.*, **25**, 467 (1956); (b) W. Moffitt and J. T. Yang, *Proc. Natl. Acad. Sci.*, **42**, 596 (1956); (c) W. Moffitt, *ibid.*, **42**, 736 (1956); (d) W. Moffitt, D. D. Fitts and J. G. Kirkwood, *ibid.*, **43**, 723 (1957).

(13) C. Cohen and A. G. Szent-Gyorgi, THIS JOURNAL, **79**, 248 (1957).

(14) I. Tinoco and R. W. Woody, *J. Chem. Phys.*, **32**, 461 (1960).

(15) E. R. Blout in C. Djerassi, "Optical Rotatory Dispersion," Chap. 17, McGraw-Hill Book Company, Inc., New York, N. Y., 1960.

(16) E. M. Bradbury, L. Brown, A. R. Downie, A. Elliott, W. E. Hanby and T. R. R. McDonald, *Nature*, **183**, 1736 (1959).

(17) Derived from the square root of the slope obtained from a plot of $[\alpha]$ *vs.* $\lambda^2[\alpha]$.