

Table I. The Helix-Coil Transition in Synthetic Polypeptides Measured by Polarization of Fluorescence Methods

Polymer	pH	p^a	$T_T, ^\circ\text{C.}$	$\rho_h^5 \times 10^8,$ sec. ^b	ρ_h^5/ρ_0^5	$V_e^5 \times 10^{-3},$ cc./mole
Poly Glu ⁹⁷ Lys ³⁶	3.27	0.185	21	5.2	0.37	26
	5.35	0.022	12	0.8	0.06	4
	9.58	0.010	<0			
Rigid sphere			None	14		71
Poly Lys (No. 2) ^d	7.20	0.053	13	0.7	0.04	4
	9.13	0.053	17	1.0	0.06	5
	10.03	0.061	30	2.4	0.15	12
	11.00	0.087				
Rigid sphere			None	16		83
Poly Glu ⁶³ Lys ³⁷ (No. 3) ^e	4.67	0.088	32	7.5	0.38	38
	7.20	0.049	10	0.9	0.05	5
	9.72	0.049	7	0.6	0.03	3
	11.00	0.044				
Rigid sphere			None	20		100

^a Measured with DNS-labeled polypeptides. ^b Measured with fluorescein-labeled polypeptides. ^c Mol. wt. 125,000. ^d Mol. wt. 105,000. ^e Mol. wt. 152,000.

polarization at pH 4 can be 85% reduced by 9 *M* urea, and this reflects destruction of the helix.

The modest increases in ρ_h^5 , ρ_h^5/ρ_0^5 , and V_e^5 in going from the coil to the helical form of poly Lys (No. 2) reflect a helical structure that is less rigid than that of poly Glu⁹⁷Lys³⁶. The midpoint of the helix-coil transition by polarization measurements (pH 9.6 in water) is approximately the same as that obtained from titration (pH 9.4 in water),¹³ but lower than that from optical rotation (pH 10.0 in water).¹³ This suggests that the helical form of poly Lys reaches its maximal rigidity before all of the residues have gone into the helical conformation.

When poly Lys (No. 2) in solution at pH 10.03 is heated and then cooled, it shows a melting out of rigid structure at 24°, a transition to a more rigid structure at 50°, and a return to the original, less rigid structure at 30°; the process is reversible. These changes in rigidity agree with the α -helix to β -conformation change observed in solid films.¹⁴

The helix-coil transition in poly Glu⁶³Lys³⁷ (No. 3) shows that the glutamic acid residues clearly form the dominant helix. The midpoint of the transition by polarization measurements (pH 5.2 in 0.2 *M* NaCl + 0.1 *M* phosphate or citrate buffer) is higher than that obtained by titration (pH 4.42 in 0.15 *M* KCl),¹⁵ but the same as that from optical rotation in polypeptides of comparable composition (pH 5.2 in 0.2 *M* NaCl¹² and water¹⁶). Thus the changes in rigidity and conformation occur at the same time. There is no evidence for a lysine helix at alkaline pH values either by polarization of fluorescence or optical rotation; in fact, both p and $[\alpha]^{12D}$ decrease. The degree of polarization of the copolymer at pH 4 can be 85% reduced by 9 *M* urea; this reflects destruction of the glutamic acid helix.

The same conclusions concerning the relationship between changes in rigidity and helical content in the different polymers can also be substantiated by com-

paring the pH values at which the maximal rigidity (polarization of fluorescence) and maximal helical content (optical rotation) occur. The pH values of maximal polarization, complete titration, and maximal optical rotation, respectively, under the same conditions used to obtain the data about the midpoints of the transitions¹⁷ are: 3.5, 2.8, and 4.5 for poly Glu⁹⁷-Lys³⁶; 10.7, 11.0, and 11.8 for poly Lys (No. 2); and 4.2, 3.0, and 4.0 for poly Glu⁶³Lys³⁷ (No. 3).

The equivalent volume of the rotating segment V_e^5 in all polypeptides is considerably larger than that of DNS plus the lysine side chain (0.25×10^3 cc./mole) or fluorescein plus the lysine side chain (0.34×10^3 cc./mole). Thus the rotational unit causing depolarization is a segment of the macromolecule and not just the dye conjugated to it.

The intrinsic viscosity of poly Lys (No. 2) at 25° in 0.2 *M* NaCl + 0.1 *M* buffer (citrate or phosphate) increases monotonically and reversibly from 0.70 dl./g. at pH 7.26 to 1.65 dl./g. at pH 0.80, but the degree of polarization of the DNS-labeled polypeptide at the same temperature and salt concentration does not change. The increase in viscosity in acid solution is largely suppressed by 3.0 *M* NaCl. This phenomenon has, most likely, an electrostatic basis and indicates that polylysine expands in acid solution.

Polarization of fluorescence is a very sensitive hydrodynamic method that is useful in measuring structural transitions, including the helix-coil transition, in polypeptides. It is of particular interest when used in conjunction with spectroscopic methods to correlate the extent of conformational change and the change in hydrodynamic properties of the molecule.

(17) References are the same as those for data on the midpoints of the transitions.

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An Authenticated Perchlorate Complex

Sir:

There has recently been much interest in compounds containing the so-called weakly coordinating ligands,

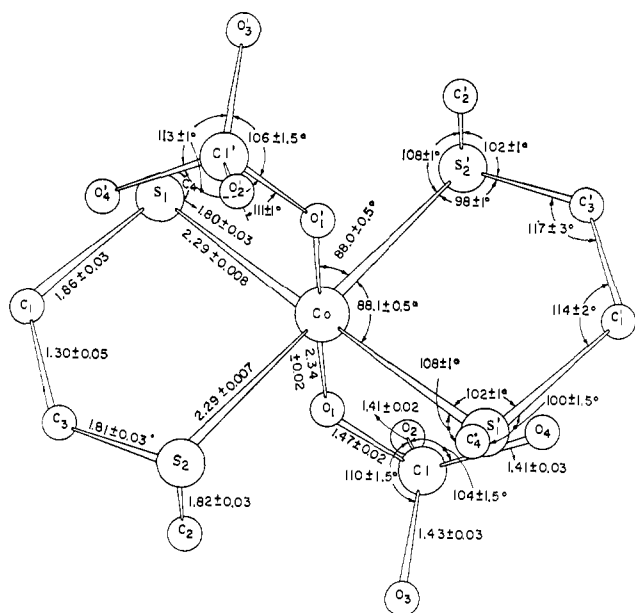


Figure 1. The structure of $\text{Co}(\text{CH}_3\text{S}(\text{CH}_2)_2\text{SCH}_3)_2(\text{ClO}_4)_2$ projected on the (010) crystal plane (bond distances in Å. and interbond angles in degrees are shown).

NO_3^- and ClO_4^- . For the nitrate ion, the weight of evidence from both chemical¹⁻³ and structural⁹⁻⁶ investigations has shown that, in fact, this is not a particularly poor ligand after all. While it still seems reasonable to consider the perchlorate ion a weak ligand, it is not clear⁷⁻⁹ that it has at least some ability to function as such, and several recent chemical and infrared studies have led to the postulation of "coordinated perchlorate ions." However, direct evidence (*i.e.*, from diffraction studies) has not, to our knowledge, ever been presented for such a claim, and surely some doubt could be said to remain regarding the nature of such compounds. We describe here the characterization, by single crystal X-ray diffraction, of an authentic perchlorate complex, bis(2,5-dithiahexane)cobalt(II) perchlorate.¹⁰

The crystallographic study gave the following results: habit, red parallelipipedon; monoclinic, $a = 8.25 \pm 0.01$, $b = 10.27 \pm 0.01$, $c = 12.11 \pm 0.01$ Å., $\beta = 115.6 \pm 0.2^\circ$, $V = 925.5$ Å.³; space group, $P2_1/c$ from systematic absences; $Z = 2$; X-ray density, 1.87 g. cm.⁻³, compared to density measured by flotation, 1.8 ± 0.1 g. cm.⁻³. A total of 650 reflections were measured, of which 125 were observed as zero, using the equi-inclination Weissenberg technique and visual compari-

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- (10) A compound to which this composition was assigned was earlier reported by Carlin and Weissberger,¹¹ but it was obtained in an impure form with a reported effective magnetic moment far different from that which we find. The compound is not very stable and purification presents some difficulties, so that in all probability the substance described by Carlin and Weissberger was an impure specimen of the compound we have studied.
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son with a set of timed exposures of one reflection. The crystal used was small and absorption corrections were neglected. The structure was solved from Patterson syntheses, refined by Fourier and least-squares methods. At present, using anisotropic temperature factors for all atoms, the discrepancy index is 13.5%.

The Co atoms lie at crystallographic centers of inversion. Figure 1 shows the structure of one formula unit and gives its principal dimensions. The most important feature for present purposes is the location of the perchlorate ions relative to the cobalt atom. The Co-O distance of 2.34 Å. indicates a well-defined bonding interaction, but since it is perhaps 0.1-0.3 Å. longer than Co-O bonds in various other Co(II) complexes, it also suggests that the perchlorate ion is a relatively weak ligand.

The infrared spectrum of the compound is in excellent agreement with the structure, along the lines previously suggested⁷⁻⁹ for a C_{3v} perturbation of the ClO_4^- ion. There are bands (cm.⁻¹) at 1115 (s, b), 1047 (s), ~925 (m, b), and ~625 (m, doublet) which can be readily assigned⁷ to the perchlorate ion coordinated as it is in this complex.

Various other features of this complex, particularly its magnetic behavior and electronic spectrum, have also been studied and a complete report is in preparation.

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Strained Systems.

IV.¹ The Tricyclo[1.1.0.0^{2,4}]butane System

Sir:

Current interest in strained systems is evident from numerous recent publications concerning syntheses and properties of these molecules.² Tricyclo[1.1.0.0^{2,4}]butane³ (tetrahedrane) is one of the most highly condensed compounds composed of carbon-carbon single bonds. The recent success⁴ in the photochemical synthesis of the tricyclo[1.1.1.0^{4,6}] system has led us to apply a similar method to the synthesis of diphenyl-tetrahedrane. We now wish to report the synthesis of this intriguing molecule.

(Δ^2 -2,3-Diphenylcyclopropenyl)carbinol⁵ was oxidized with chromic anhydride in pyridine to afford in 90% yield the corresponding aldehyde (I), m.p. 103.5-104°. *Anal.* Found: C, 87.11; H, 5.50. Compound I was converted to its tosylhydrazone (II), m.p. 196-198°. *Anal.* Found: C, 71.27; H, 5.13; N, 7.42. A solution of II (500 mg.) in dry tetrahydrofuran (200 ml.) containing an equivalent amount of sodium methoxide was irradiated at -20° under an inert atmosphere with a Hanovia 450-w. mercury lamp using

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