260.   *Polypeptides.   Part X.*[1]   *The Optical Rotatory Dispersion of Poly-γ-D-glutamic Acid.*

By H. N. RYDON.

The optical rotatory dispersion of poly-γ-D-glutamic acid, from *Bacillus anthracis*, has been measured from 250 to 600 mμ.   The dispersion is simple in alkaline solution but complex, obeying the Moffitt equation, in acid solution.   It is suggested that the un-ionised acid possesses a helical structure in solution, and two possible structures are described.

THE polypeptide,[2,3] which is the major constituent of the capsule of *Bacillus anthracis*, is remarkable in being made up entirely of D-glutamic acid residues[3] joined together by γ-peptide linkages.[4]   The extensive physicochemical investigations by Kent, Record, and Wallis[5] and by Edelhoch and Bateman[6] revealed considerable conformational differences, in solution, between the (un-ionised) free acid and the (ionised) sodium salt.   Studies of

[1] Part IX: Hanson and Rydon, *J.*, 1964, 836.
[2] Bruckner and Ivánovics, *Z. physiol. Chem.*, 1937, **247**, 281; Ivánovics and Erdös, *Z. Immunitäts.*, 1937, **90**, 5; Ivánovics and Bruckner, *ibid.*, 1937, **90**, 304; 1938, **93**, 119.
[3] Hanby and Rydon, *Biochem. J.*, 1946, **40**, 297.
[4] Kovács and Bruckner, *J.*, 1952, 4255; Bruckner, Kovács, and Nagy, *J.*, 1953, 148; Kovács, Bruckner, and Kovács, *J.*, 1953, 145; Bruckner, Kovács, and Kovács, *J.*, 1953, 1512; Bruckner, Kajtár, Kovács, Nagy, and Wein, *Tetrahedron*, 1958, **2**, 211.
[5] Kent, Record, and Wallis, *Phil. Trans.*, 1957, **250**, *A*, 1.
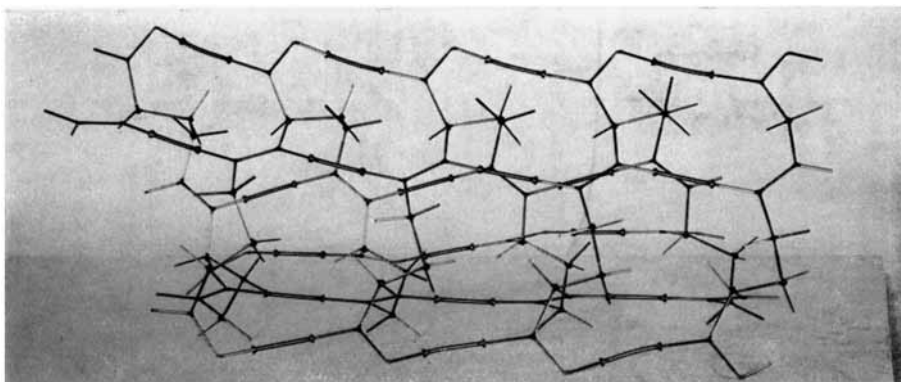[6] Edelhoch and Bateman, *J. Amer. Chem. Soc.*, 1957, **79**, 6093.

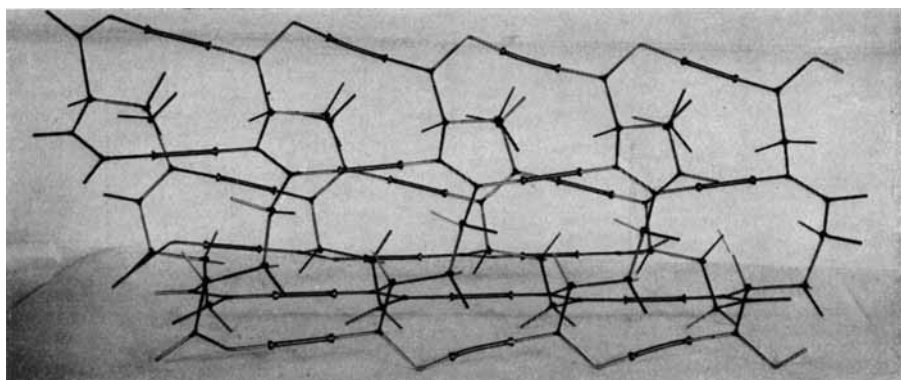Plate A. Dreiding model of poly-γ-D-glutamic acid 3_{17} helix.

PLATE B. Dreiding model of poly-γ-D-glutamic acid 3_{19} helix.

optical rotatory dispersion have been much used in recent years to elucidate conformational changes in proteins and polypeptides in solution [7] and it seemed likely that such studies would lead to useful results in the present case.

Such an investigation was carried out recently by Edelhoch and Lippoldt,[8] who found the optical rotatory power of the polypeptide to be markedly pH dependent, being positive in acid, and negative in alkaline, solution. These authors describe the optical rotatory dispersion as simple at all pH values, but their results are difficult to interpret, since at pH values corresponding to 30—50% ionisation the Drude dispersion constant, λ_c, is imaginary, which is physically meaningless. Edelhoch and Lippoldt worked only from 700 to 400 mμ and it seemed desirable to extend their observations to lower wavelengths.

In the present work the optical rotatory power of poly-γ-D-glutamic acid has been measured, in aqueous solutions of both the free acid and its sodium salt, at a series of wavelengths between 600 and 250 mμ. Fig. 1 shows the modified Drude plot [9] of the results. It will be seen that the results for the sodium salt (curve A) yield a satisfactory straight line, showing them to conform to the simple Drude equation *

$$[\alpha]_\lambda = a_c\lambda_c^2/(\lambda^2 - \lambda_c^2) \tag{1}$$

with the parameters given in the Table. The results for the free acid (curve B), however, give the type of curve characteristic of substances exhibiting complex dispersion conforming to the Moffitt equation.[10,11] The upper branch of curve B in Fig. 1 corresponds to values of λ down to 320 mμ; Edelhoch and Lippoldt [8] worked only down to 400 mμ and it is clear that they were misled, by the approximate linearity of this part of the curve, into assuming the dispersion to be simple; the negative, or zero, slopes ($= \lambda_c^2$) of this branch of their curves account for the imaginary and zero values of λ_c assigned by them between 30 and 50% and between 0 and 30% ionisation, respectively.

Fig. 2 shows the Moffitt plot [11] for the free acid, with $\lambda_0 = 197$ mμ; the plot is satisfactorily linear showing the rotatory dispersion of the free acid to conform to the Moffitt equation:

$$[\alpha]_\lambda = a_0\lambda_0^2/(\lambda^2 - \lambda_0^2) + b_0\lambda_0^4/(\lambda^2 - \lambda_0^2)^2 \tag{2}$$

with the parameters given in the following Table, in which the corresponding parameters for poly-α-L-glutamic acid [9] are included for comparison.

Optical rotatory dispersion of polyglutamic acids.

	Ionised (Drude)		Un-ionised (Moffitt)		
	a_c	λ_c	a_0	b_0	λ_0
Poly-γ-D-	$-111°$	222 mμ	$+295°$	$-226°$	197 mμ
Poly-α-L-	$-517°$	212 mμ	$+120°$	$-625°$	212 mμ

The experimentally determined values of $[\alpha]_\lambda$ are plotted against λ in Fig. 3, in which the lines have been drawn from equations (1) and (2), with the parameters given in the Table. It will be seen that the agreement between the observed and calculated values of $[\alpha]$ is very satisfactory.

Mason [12] has recently re-validated the Moffitt equation and the change from Moffitt to Drude behaviour of the optical rotatory dispersion of poly-γ-D-glutamic acid on ionisation, which is similar to that observed with many α-linked polypeptides on passing from

* a_c is used instead of the conventional a_0, to avoid confusion with the similar parameter in the first term of the Moffitt equation.

[7] Cf. Urnes and Doty, *Adv. Protein Chem.*, 1961, **16**, 401.
[8] Edelhoch and Lippoldt, *Biochim. Biophys. Acta*, 1960, **45**, 205.
[9] Yang and Doty, *J. Amer. Chem. Soc.*, 1957, **79**, 761.
[10] Moffitt, *J. Chem. Phys.*, 1956, **25**, 467; *Proc. Nat. Acad. Sci. U.S.A.*, 1956, **42**, 736.
[11] Moffitt and Yang, *Proc. Nat. Acad. Sci. U.S.A.*, 1956, **42**, 596.
[12] Mason, *Quart. Rev.*, 1963, **17**, 20; *Nature*, 1963, **199**, 139.

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the α-helical to the random-coil structure,[7] is most reasonably interpreted as resulting from a similar change from an ordered structure, with some inherent, structural, rotatory power in addition to that due to the asymmetric carbon atoms, to a disordered one, in which the rotatory power is due only to the asymmetric carbon atoms.
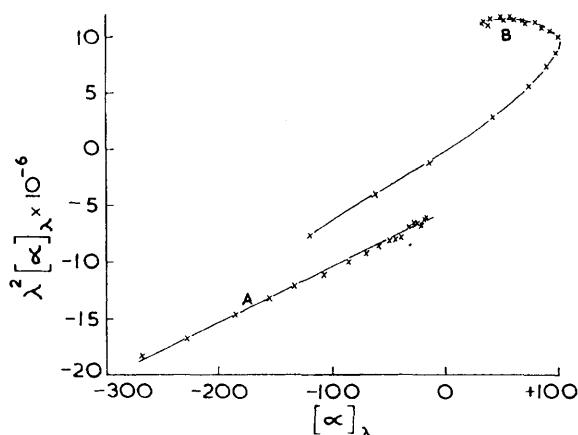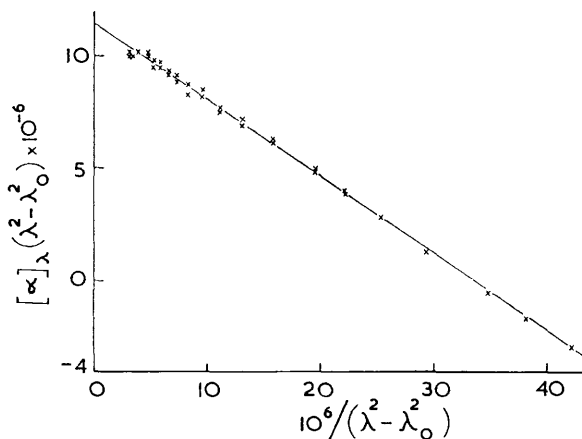

FIG. 1.   Modified Drude plots for poly-γ-D-glutamic acid.

Curve A, sodium salt; curve B, free acid.

FIG. 2.   Moffitt plot for poly-γ-D-glutamic acid.

Edelhoch and Lippoldt [8] studied the effect of the variation of pH, between 2·9 and 7·2, on the optical rotatory dispersion of poly-γ-D-glutamic acid. The fraction, f_R, of randomly coiled structure at various pH values has been calculated from their results, using the interpolation formula:

$$[\alpha]_\lambda = f_R \left(\frac{a_c \lambda_c^2}{\lambda^2 - \lambda_c^2} \right) + (1 - f_R) \left(\frac{a_0 \lambda_0^2}{\lambda^2 - \lambda_0^2} + \frac{b_0 \lambda_0^4}{(\lambda^2 - \lambda_0^2)^2} \right)$$

with the values of the constants given in the Table. The results are plotted in Fig. 4; the point of inflexion is at pH 4·0, in good agreement with the pK_a (4·06) determined [3] by direct electrometric titration. This behaviour is similar to that shown by poly-α-L-glutamic acid [13] and may similarly be ascribed to breakdown of the ordered, helical structure on ionisation.

[13] Doty, Wada, Yang, and Blout, *J. Polymer Sci.*, 1957, **23**, 851; Idelson and Blout, *J. Amer. Chem. Soc.*, 1958, **80**, 4631; Goldstein and Katchalski, *Bull. Res. Council Israel*, 1960, **9A**, 138.

Poly-γ-D-glutamic acid cannot, of course, exist in the α-helical form [14] common to so many α-polypeptides, but examination of Dreiding models shows that other helical

FIG. 3. Optical rotatory dispersion of poly-γ-D-glutamic acid.
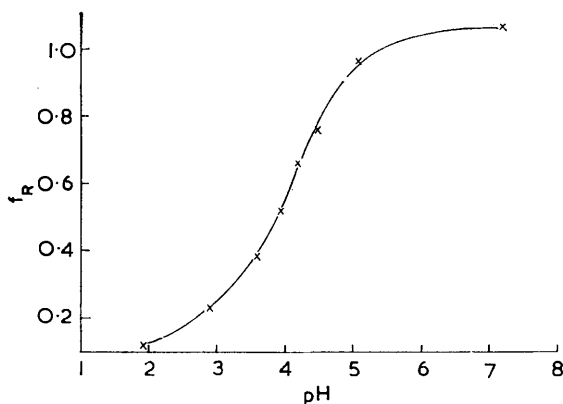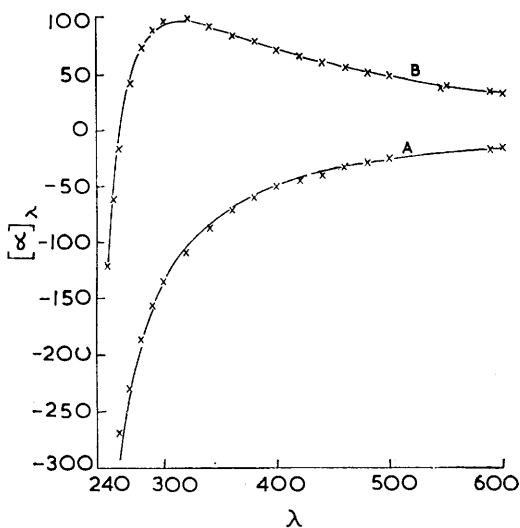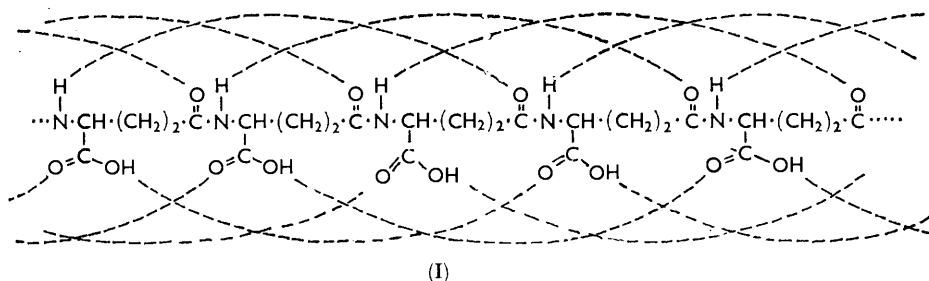
Curve A, sodium salt; curve B, free acid.

FIG. 4. Helix-random coil transition in poly-γ-D-glutamic acid.

structures are possible; the two most satisfactory of these (3_{17} and 3_{19}, according to the nomenclature of Bragg, Kendrew, and Perutz [15]) are discussed below.

The 3_{17} helix (Plate A and Fig. 5) results from suitable coiling of structure (I), in

(I)

[14] Pauling, Corey, and Branson, *Proc. Nat. Acad. Sci. U.S.A.*, 1951, **37**, 205.
[15] Bragg, Kendrew, and Perutz, *Proc. Roy. Soc.*, 1950, **203**, *A*, 321.

which 17-membered rings are generated by hydrogen bonding of the NH of every nth peptide group to the CO of the $(n + 3)$rd. The model with free α-carboxyl groups collapses very easily, but hydrogen-bonding of these stiffens it and makes it quite rigid. This structure, with three residues and a translation of 4·8 Å per turn, seems very satisfactory; as will be seen from Plate A, the hydrogen atoms attached to the β- and γ-carbons have the staggered conformation. The structure illustrated is the left-handed helix; the
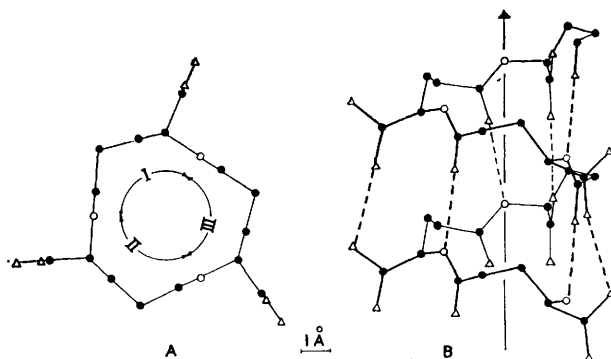

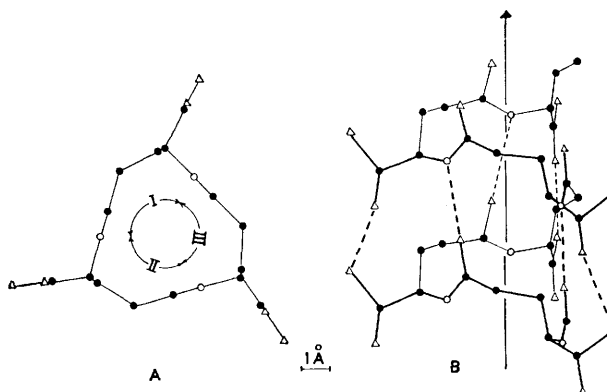FIG. 5. Poly-γ-D-glutamic acid 3_{17} helix.

A, Plan; B, elevation (thick lines are bonds in front of plane, thin lines bonds behind plane, broken lines hydrogen bonds).

● = carbon; △ = oxygen; ○ = nitrogen.

FIG. 6. Poly-γ-D-glutamic acid 3_{10} helix.

A, Plan; B, elevation (thick lines are in front of plane, thin lines bonds behind plane broken lines hydrogen bonds).

● = carbon; △ = oxygen; ○ = nitrogen.

right-handed helix is not satisfactory, the carboxyl groups being directed inwards in a manner unfavourable for hydrogen-bonding. The negative value of the Moffitt coefficient, b_0, may be regarded as supporting a left-handed helical structure. In the 3_{17} helix the amide transition dipoles lie more nearly parallel ($v = 25°$) to the axis of the helix than in the α-helix ($v = 50°$); [10] in consequence, since $v < 45°$, the absorption due to the parallel transition, arising from coupling of the individual group moments, lies at

(II)
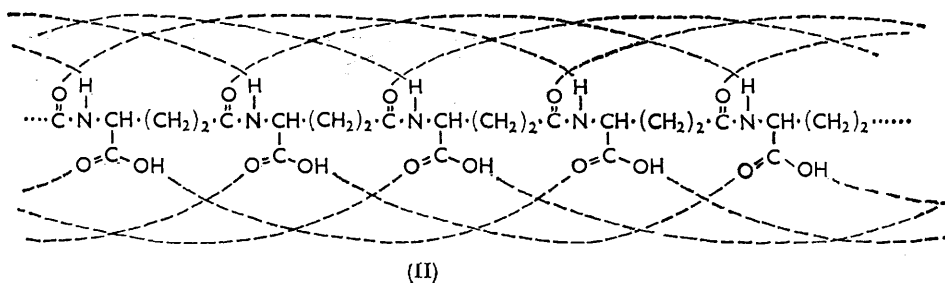
a shorter wavelength than that due to the perpendicular transition. This is the converse of the situation with the α-helix, and b_0, which is positive for a left-handed α-helix, should therefore be negative for a left-handed 3_{17} helix.

In the 3_{19} helix (Plate B and Fig. 6) the generating structure is (II), in which 19-membered rings arise by hydrogen bonding of the CO of every nth peptide group to the NH of the $(n + 3)$rd. As with the 3_{17} helix the model is stiffened by hydrogen-bonding of the α-carboxyl groups and only the left-handed helix (illustrated) places them in a favourable arrangement for this. This structure also has three residues and a translation of 4·8 Å per turn. It is a little less satisfactory than 3_{17} since, as can be seen from Plate B, the β- and γ-hydrogen atoms are almost eclipsed.

The collapse of the models when the inter-carboxyl hydrogen bonds are broken provides a very satisfying mechanical analogy for the collapse of the ordered structure when poly-γ-D-glutamic acid ionises and it is suggested that it is indeed the breaking of these inter-carboxyl hydrogen bonds which is responsible for the transition from the helical to the random-coil conformation on raising the pH. The presence of these inter-carboxyl hydrogen bonds in the un-ionised molecule also provides a satisfactory explanation of the weakness of the α-carboxyl groups in poly-γ-D-glutamic acid (pK_a 4·06) as compared with those in glutamyl-glutamic acid (pK_a 2·90),[3] glycyl-glutamine (2·88),[16] leucyl-glutamine (2·99)[16] and peptides generally (3·0—3·2).[17] Poly-α-glutamic acid is likewise weaker (pK_a 5·5—6·0)[13] than would be expected from the known pK_a's for the γ-carboxyl group in glutamic acid (4·25), glutamyl-glutamic acid (4·35),[3] isoglutamine (3·81),[16] and glutaminyl-glutamic acid (4·38),[16] and it seems likely that this difference, too, may be due to inter-carboxyl hydrogen bonding; it is of interest that Yang[18] has recently suggested such bonding as a possible factor in stabilising the α-helix in un-ionised poly-α-glutamic acid.

This work is being extended to other ω-polypeptides derived from other α-amino-dicarboxylic acids (*e.g.*, aspartic and α-aminoadipic acids).

EXPERIMENTAL

The poly-γ-D-glutamic acid used was sample H230B of Hanby and Rydon;[3] concentrations ($c = 0·8$—$1·4$ g./100 ml.) were corrected for the water and ash contents of the specimen.

Optical rotations were measured at room temperature in water (free acid) or 0·1N-sodium hydroxide (sodium salt) with a Bellingham and Stanley " Peepol " spectropolarimeter using a cell-length of 0·5 dm. The experimental points (Fig. 3) are mean values from two independent experiments in each case.

[16] Melville and Richardson, *Biochem. J.*, 1935, **29**, 187.
[17] Cohn and Edsall, " Proteins, Amino-acids, and Peptides as Ions and Dipolar Ions," Reinhold New York, 1943, Ch. IV.
[18] Yang, *Tetrahedron*, 1961, **13**, 143.