LETTERS TO THE EDITORS

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Absolute Configuration and Optical Rotation of Folded (α) Polypeptides

Since Pauling and Corey[1] proposed the α-helix for the structure of some folded polypeptides, evidence for the essential correctness of this structure has accumulated; but agreement between the observed X-ray diffraction pattern of oriented fibres and that calculated from the model has not been obtained. As Brown and Trotter[2] have recently shown, with α poly-L-alanine ($(CO.CH.CH_3.NH)_n$), where the scattering centre of the side-chain is fixed with respect to the helix, the agreement along the layer lines is very poor. In this case, the X-ray reflexions can be indexed on a unit cell through which only one chain passes, and it was assumed that both the sense of the helix (right- or left-handed) and the direction of the peptide sequence were identical for all chains. The fit was so poor that it could not be concluded with certainty whether the helices were right- or left-handed.

Since the α-helix conforms accurately to the conditions for minimum energy and also has dimensions which are almost exactly those required to fit the unit cell of poly-L-alanine, it seems likely that the atomic parameters of this helix must be very nearly correct. We have accordingly examined more general arrangements of eighteen-residue, five-turn helices (fibre repeat 27 A.), using the reflecting optical diffraction spectrometer which was described recently[3]. The fibre repeat in poly-L-alanine is longer than this[2], but the effect of the longer repeat on the optical transform is not appreciable. Like-handed hexagonal arrangements of helices which have near six-fold screw axes pack with the observed inter-chain distance whether all chains have the same direction of peptide sequence or not; the necessary condition is that the β-carbon atoms of the methyl side-chain groups should be in identical crystallographic positions. A random arrangement of the direction of the peptide sequence of the individual chains (which is very probable in the absence of strong polarizing forces) will produce the diffraction pattern of a primitive unit cell, though there is, in the strict sense, no unit cell. This arrangement of right-handed helices (corresponding to βC_1[4]) gives diffraction patterns in much better agreement with the observed X-ray pattern than does a left-handed one. The refinement of co-ordinates is not yet complete, but enough has been done to show that satisfactory agreement may be expected with quite minor changes in the co-ordinates originally given by Pauling and Corey[5]. It is most unlikely that any appreciable proportion of left-handed helices contributes to the crystallite reflexions. It is possible, however, that left-handed helices contribute to the observed layer-line streaks; but in this case the proportion would be small. We believe, therefore, that in poly-L-alanine the helices are mainly right-handed, and presumably α-helices of other L-amino-acid polymers will also be right-handed.

The specific optical rotation of an α-helix of poly-glycine (using a very simplified model) has recently been calculated by Fitts and Kirkwood[6]; for right-handed helices this is found to be +132°, in an aqueous solution with index of refraction 1·35. Poly-L-(or poly-D)-alanine is not soluble in non-polar solvents, and since in polar solvents the chains may be random coils (at least at low polymer concentrations) a direct measurement of the specific rotation of an α-helix of poly-L-alanine cannot well be made. However, a *meso* polymer containing a small proportion of one enantiomorph is slightly soluble in chloroform. From measurements on such material, the specific rotation of poly-L-alanine is about +50°, which is considerably less than the figure given by Fitts and Kirkwood for a right-handed helix without active side-groups. Unless their estimate is grossly in error, it would appear that the contribution of the side-chain opposes the form optical rotation of the helix. This would account for the generally low value of the specific rotation of polypeptides in conditions which favour the α-helix form. Fitts and Kirkwood[7] have recently, from similar considerations of optical rotation, also concluded that α-polypeptides form right-handed helices.

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An Improvement of the Hydrazine Method for Determination of C-Terminal Amino-acids

Hitherto, investigators using the hydrazine method of Akabori and co-workers[1] found it necessary to treat the peptides or protein with anhydrous hydrazine at not less than 100° for about 10 hr. in order to break all peptide bonds[2-4]. This severe treatment causes complete decomposition of cysteine, cystine and poor yields of arginine, aspartic acid and glutamic acid, with the result that they cannot be determined satisfactorily as C-terminal amino-acids. It is now shown that by the addition of an acid catalyst it is possible to decompose peptides under much milder conditions so that all the common amino-acids can be recovered in fair yield.

About 10 mgm. peptide or amino-acid and 26 mgm. $N_2H_4H_2SO_4$ were dried for about 3 hr. at room temperature under vacuum conditions (about 10^{-4} mm. mercury), 0·2 ml. 100 per cent N_2H_4 distilled in, the tube sealed, heated at 60° for 16 hr. and the excess hydrazine evaporated off over concentrated sulphuric acid in a vacuum desiccator. The residue was dissolved in about 1 ml. dilute hydrochloric acid, shaken with 0·4 ml. benzaldehyde for 2 hr., the aqueous solution separated by centrifugation and dinitrophenylated by Levy's method at 40° in *M*/2 carbonate buffer[5,6]. The aqueous solution (about 8 ml.) was extracted with ether (4 × 5 ml.), acidified with hydrochloric acid and extracted five times with 5 ml. ethyl acetate. The dinitrophenyl-amino-acids and unchanged peptides were separated by one-dimensional paper chromatography using the toluene,

phosphate and *t*-amyl alcohol solvents[7]. (With lysozyme it was necessary to remove the dinitrophenol by vacuum sublimation at 40° prior to one- and two-dimensional chromatography). The spots were cut out, eluted with 1 per cent sodium bicarbonate and the optical density measured at 360 mμ (385 mμ for dinitrophenyl-proline) with a Unicam spectrophotometer. These values were converted to percentage yields (accuracy ± 10 per cent) using the results from dinitrophenylation, extraction and chromatography of each amino-acid or peptide separately.

From Fig. 2 it is clear that the hydrazinolysis of DL-leucylglycine is virtually complete after 16 hr. at 60° in hydrazine containing 1·0 *M* hydrazine sulphate but requires 80° for 16 hr. in the absence of catalyst. Since the yields of glycine are approximately the same by both methods (see Fig. 1) the milder acid-catalysed reaction is of no advantage in this particular case. However, it is to be preferred in general because of the greatly increased stability of some of the amino-acids at 60°. From Table 1 it is clear that all

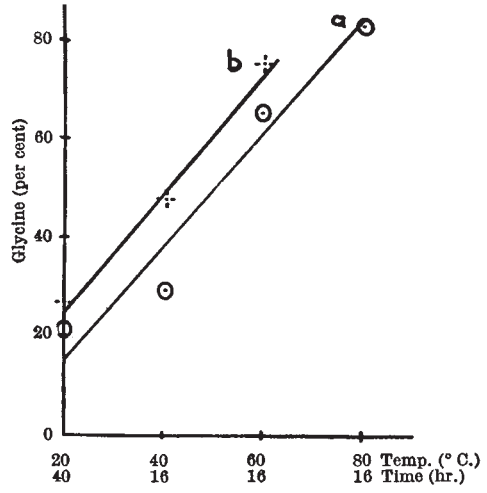

Fig. 1. Yield of glycine on hydrazinolysis of DL-leucylglycine: *a*, no catalyst; *b*, 1·0 *M* $N_2H_4H_2SO_4$

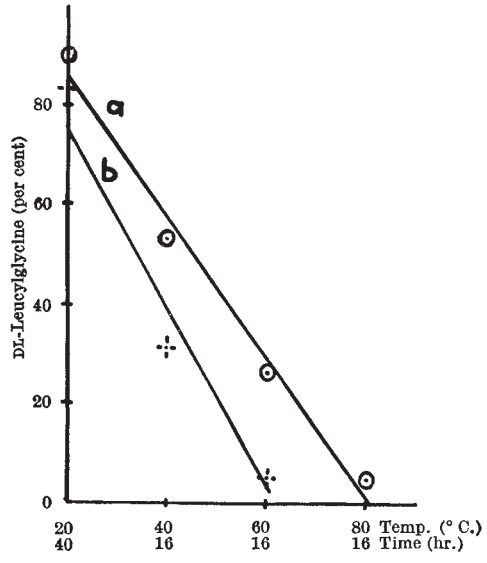

Fig. 2. Yield of unchanged DL-leucylglycine on hydrazinolysis of DL-leucylglycine: *a*, no catalyst; *b*, 1·0 *M* $N_2H_4H_2SO_4$

Table 1. YIELD OF C-TERMINAL AMINO-ACID OR AMINO-ACID ON HYDRAZINOLYSIS OF PEPTIDES AND AMINO-ACIDS

Material	C-Terminal amino-acid (%)	
	60°—16 hr. 1·0 *M* $N_2H_4H_2SO_4$ in N_2H_4[a]	100°—8 hr. N_2H_4, Niu and Fraenkel-Conrat (ref. 3)
L-Arginine hydrochloric acid	60[b]	25[b]
L-Aspartic acid	99	19
DL-Cysteine hydrochloric acid	86[c]	0
L-Cystine	78	0
L-Glutamic acid	76	16
DL-Histidine	53	43
L-Lysine hydrochloric acid	90	47
DL-Methionine { met.	66	0
DL-Methionine { met. sulphoxide	35	56
DL-Proline	97	52
DL-Serine	96	39
DL-Threonine	105	52
DL-Tryptophan	46	39
DL-Alanylglycine	92	39
DL-Glycylleucine	69	
DL-Glycylphenylalanine	98	
L-Glycyltryptophan	20[d]	
L-Glycyltyrosine	65	
DL-Leucylglycine	75	
Glutathione	91 gly	

a, Yield of unchanged peptide always < 5 per cent; *b*, L-arginine completely destroyed and determined as ornithine (ref. 3); *c*, DL-cysteine completely oxidized to cystine and determined as such; *d*, 56 per cent yield in absence of $N_2H_4H_2SO_4$, hence the breakdown of tryptophan must be catalysed by acid.

the reactive amino-acids, except tryptophan and histidine, are recovered in much better yields, particularly arginine, aspartic acid, cysteine, cystine and glutamic acid. A number of peptides are included to show the application of the method in this field. A 45 per cent yield of leucine was obtained from lysozyme (molecular weight 14,900), and further work on proteins, which is now in progress, will be reported later.

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A New Antagonist of Thiamine

4-AMINO-5-hydroxymethyl-2-methylthiopyrimidine[1,2] (I) is an inhibitory antagonist of the pyrimidine moiety of vitamin B_1 (II)[3]. However, a thiamine-requiring mutant of *Escherichia coli* (strain *M*70–17) which can use II for growth will grow in the presence of I if the thiazole moiety of thiamine is also supplied. One possible explanation of this is that, under these conditions, the pyrimidine I is being converted to a functional thiamine-like compound. Consequently, it was decided to synthesize the thiamine analogue derived from I, which would differ from thiamine only in the nature of the 2-substituent, and