structure the K atoms are apparently too big for this arrangement. The Hg layers have been distorted, buckling in such a way that a slightly zigzag chain of K atoms can be accommodated in Na atom type holes.

As might be expected for intermetallic compounds which are exothermic upon formation, the interatomic distances between the atoms, particularly the K–K and K–Hg distances, are shorter by about 10% than the values calculated from the pure metals. In this way the K–Hg compounds are similar to the Na–Hg compounds reported previously.

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The Structure of Tussah Silk Fibroin*

(with a note on the structure of β-poly-L-alanine)

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A detailed structure for Tussah silk fibroin has been derived which is in agreement with the X-ray diffraction data. The structure is similar to that of *Bombyx mori* fibroin in that it is based on antiparallel-chain pleated sheets; the method of packing of the sheets, however, is quite different. This difference in packing can be explained on the basis of the chemical compositions of the two silks.

It seems highly probable that the structure of the β (stretched) form of poly-L-alanine is essentially that derived for Tussah silk.

Introduction

A detailed structure for commercial silk fibroin (*Bombyx mori*) has recently been formulated in these Laboratories (Marsh, Corey & Pauling, 1955). A prominent feature of the structure of *Bombyx mori* silk fibroin is the occurrence of glycine as alternate residues along the polypeptide chains.

Another form of silk fibroin is that derived from Tussah silk (commonly called wild silk). Previous investigators (Kratky & Kuriyama, 1931; Trogus & Hess, 1933) have shown that the X-ray diffraction pattern of Tussah silk fibroin, although having many features in common with the pattern obtained from *Bombyx mori*, is significantly different in several respects. Its chemical composition also differs from that of *Bombyx mori* in a very significant way (Table 1). The most striking differences are in the relative amounts of glycine and alanine. In particular, the amount of glycine in Tussah silk (26·6 residue %) is insufficient to permit the occurrence of glycine as alternate residues along the polypeptide chains. In

Table 1. *Composition of the fibroins of* Bombyx mori *and Tussah silks**

Amino-acid residue	*Bombyx mori* (residue %)	Tussah silk (residue %)
Glycine	44·4	26·6
Alanine	30·2	44·2
Serine	11·9	11·8
Tyrosine	4·9	4·9
Aspartic acid	1·4	4·7
Arginine	0·4	2·6
Valine	2·1	0·6
Glutamic acid	0·9	0·8
Tryptophan	0·2	1·1
Phenylalanine	0·6	0·5
Isoleucine	0·5	0·4
Leucine	0·5	0·4
Histidine	0·2	0·8
Proline	0·4	0·3
Threonine	1·0	0·1
Lysine	0·3	0·1
Cystine	0·1	—
Mean residue weight	78·3	83·5

* Calculated from the data of Schroeder & Kay (1955).

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contrast to the smaller percentage of glycine, the percentage of alanine is much larger in Tussah silk than in *Bombyx mori*. It seems reasonable to expect that the structural differences between the two silks, as evidenced by the differences in their X-ray patterns, are related in a simple way to these striking differences in their chemical compositions.

In the current investigation, a detailed structure for Tussah silk fibroin has been derived which is compatible with both chemical and X-ray data. This structure also has strong implications concerning the structure of the β (stretched) form of poly-L-alanine.

Experimental

Samples of Tussah silk (*Antherea pernyi*) were kindly supplied by Prof. S. Mizushima of the University of Tokyo. For X-ray photography, degummed fibers (Schroeder & Kay, 1955) were arranged in the form of a bundle about 0·5 mm. in diameter. Diffraction photographs were taken in an evacuated 3-cm.-radius camera and in a helium-filled 10-cm.-radius camera with nickel-filtered copper $K\alpha$ radiation (λ=1·5418 Å).

Standard fiber photographs were taken with the X-ray beam perpendicular to the axis of the fibers; a typical photograph is reproduced in Fig. 1, together

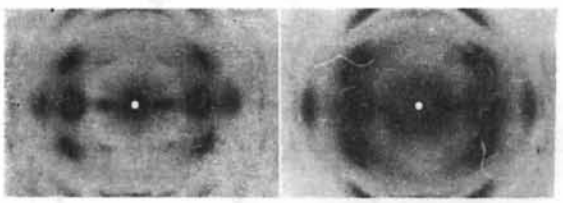


Fig. 1. X-ray diffraction photographs of *Bombyx mori* (left) and Tussah (right) silk fibroins, prepared with Cu $K\alpha$ radiation in a cylindrical camera. The fiber axes are vertical.

with a photograph of *Bombyx mori* fibroin. In addition, 6° oscillation photographs were prepared in which the axis of oscillation was perpendicular to the fiber axis; at the center of oscillation, the X-ray beam made an angle of 48° with the fiber axis. By this means the strong sixth-order meridional reflection at 1·16 Å was brought into reflecting position. For calibration purposes, the powder spectrum of sodium fluoride was superimposed on this pattern; the lattice parameter of the NaF sample was obtained separately from measurements of Straumanis-type powder photographs.

It was found that, within the limits of visual observation, the 400 reflection of NaF was exactly coincident with the center of the sixth-order meridional reflection of Tussah silk. The spacing of the 400 reflection of NaF, as measured on the Straumanis photographs, was 1·158 Å. In confirmation of this spacing, the lattice parameter a_0 was obtained from a least-squares treatment of all of the observed powder lines; it was found to be 4·6327 Å with a probable error of 0·0003 Å, in satisfactory agreement with the value 4·620±0·004 kX. (= 4·629±0·004 Å) obtained by Davey (1923). Accordingly, the fiber-axis identity distance of Tussah silk is 6·949 Å, with an estimated limit of error of about 0·010 Å.

The value 6·95 Å for the fiber-axis identity distance is slightly smaller than that found for *Bombyx mori*—6·97±0·03 Å (Marsh *et al.*, 1955). As a confirmation of this shortening, a corresponding oscillation photograph of *Bombyx mori* was prepared with the powder pattern of NaF superimposed. On this photograph, the sixth-order meridional reflection occurred at a significantly larger spacing than the 400 reflection from NaF; the fiber-axis identity distance for *Bombyx mori* was calculated to be 6·988±0·015 Å. Thus there is a real, though small, difference in the fiber-axis identity distances of the two types of silk fibroin. This difference was first reported by Bamford, Brown, Elliott, Hanby & Trotter (1953); the identity distances given by them are 6·92 Å and 6·94 Å for Tussah and *Bombyx mori*, respectively.

The measured spacings and visually-estimated intensities of the equatorial reflections from Tussah silk are listed in Table 2; these values are in good agreement with those reported by Trogus & Hess (1933). For comparison, the spacing and intensity data for *Bombyx mori* are also listed in Table 2. Intensity and spacing data for non-equatorial reflections are given in Table 5, together with the intensities calculated on the basis of the proposed structure.

Derivation of the structure

A comparison of data in Table 2 shows that the reflections which, in the case of *Bombyx mori*, have been

Table 2. *Spacings and relative intensities for equatorial reflections for* Bombyx mori *and Tussah silk fibroins*

	Tussah				*Bombyx mori*			
No.	d_o (Å)	I	$h0l$	d_c (Å)	No.	d_o (Å)	I	$h0l$
					1	9·70±0·25	90	001
1	5·35±0·10	*vvs*	002	5·30	2	4·70±0·20	450	002
2	4·35±0·08	*vs*	201	4·31	3	4·25±0·15	900	201, 20$\bar{1}$
3	2·64±0·05	*s*	004	2·65	4	3·05±0·02	180	003
4	2·36±0·04	*mw*	400	2·36	5	2·35±0·01	20	400
					6	2·10±0·05	*vw*	402, 40$\bar{2}$
					7	1·80±0·05	*vvw*	005
5	1·57±0·02	*w*	601	1·56	8	1·56±0·01	18	601, 60$\bar{1}$
					9	1·20±0·05	*vw*	800

indexed as $h00$ or $h01$ occur with very similar spacings and intensities in the two types of silk; reflections of the type $00l$, however, do not compare. The indexing of the *Bombyx mori* pattern was accomplished unambiguously from data obtained from doubly-oriented specimens, and it has been shown (Kratky & Kuriyama, 1931; Marsh *et al.*, 1955) that the $h00$ reflections arise from sets of diffraction planes oriented perpendicular to the plane of rolling of the specimens. The plane of rolling has been identified with the plane of the antiparallel-chain pleated sheets which are the basic structural feature of *Bombyx mori*, and the a axis lies within the plane of the sheets. On the other hand, the $00l$ reflections arise from sets of diffraction planes oriented perpendicular to the plane of rolling of the doubly-oriented specimens, and the c axis is an identity distance perpendicular to the pleated sheets.

Thus, the two identity distances which, in the case of *Bombyx mori*, have been shown to lie within the plane of the pleated sheets—the b-axis (fiber axis) identity distance, which represents the distance between alternate residues along the polypeptide chains, and the a-axis identity distance, which represents the distance between alternate polypeptide chains in the hydrogen-bonded sheet—appear to have close counterparts in Tussah silk. On the other hand, the methods of packing of the sheets, as represented by the $00l$ reflections, are apparently different in the two silks.

It should be pointed out that in both silk fibroins the $00l$ reflections are quite diffuse, whereas the $h00$ and $h01$ reflections are relatively sharp. This is readily understandable in terms of a pleated-sheet structure: the strong hydrogen bonding within the sheets would be expected to lead to well-defined repeat distances and, hence, sharp reflections in the direction of the a axis, whereas any disorder in the packing of adjacent sheets would result in diffuse reflections in the direction of the c axis.

With the aid of the *Bombyx mori* pattern, we have been able to index the diffraction pattern of Tussah silk on the basis of an orthogonal unit cell with

$$a_0 = 9{\cdot}44,\ b_0 \text{ (fiber axis)} = 6{\cdot}95,\ c_0 = 10{\cdot}60 \text{ Å}:$$

the corresponding values for the pseudo unit of *Bombyx mori* are 9·40, 6·97 and 9·20 Å, respectively. The indexes and calculated spacings of the equatorial reflections listed in Table 2 are based on this unit cell. This cell will account for all of the reflections observed for Tussah silk, both equatorial and non-equatorial, as is shown subsequently in Table 5. Accordingly, we have chosen it as a unit of structure, bearing in mind that, as was the case for *Bombyx mori*, it is too small to accommodate the larger amino-acid residues known to be present in the protein, and hence must be regarded only as a pseudo unit cell.

Prof. R. Brill has called to our attention that the dimensions of this orthorhombic unit cell of Tussah silk are essentially equivalent to those proposed by him (Brill, 1943) for a monoclinic unit cell for Satonia-type silk.

In the formulation of positional parameters for all of the atoms within this pseudo unit cell, we have assumed that the basic structural component is the antiparallel-chain pleated sheet (Pauling & Corey, 1953). The reasons for this choice have been discussed in connection with *Bombyx mori*; they are founded principally on the confidence we place in our knowledge of the geometry of polypeptide chains and hydrogen bonds. In particular, the values for the a- and b-axis identity distances—9·44 and 6·95 Å—are almost exactly those calculated for the antiparallel-chain pleated sheet—9·5 and 7·0 Å (Pauling & Corey, 1953).

The formulation of the detailed structure of Tussah silk was arrived at by a consideration of the ways in which adjacent pleated sheets might pack together. The absence of odd orders of reflections of the type $00l$ and the relatively high intensities of the 002 and 004 reflections indicate that the pleated sheets are spaced at approximately equal intervals of 5·3 Å along the c axis; the relative position of adjacent pleated sheets was determined by packing considerations.

The pseudo unit of structure of Tussah silk is shown diagrammatically in Fig. 2; the positional parameters

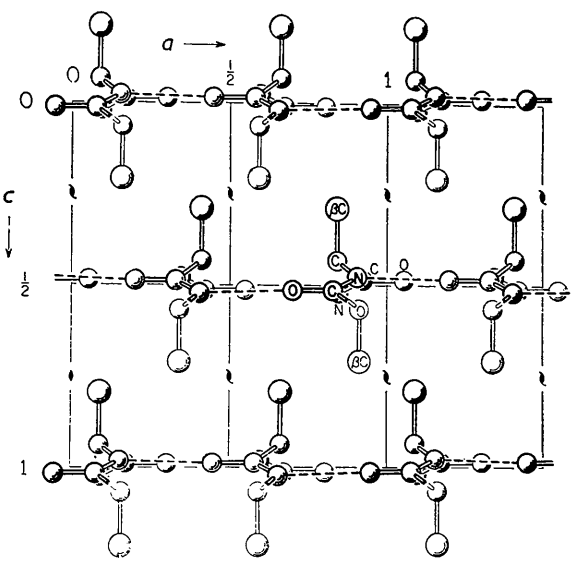


Fig. 2. A representation of the pseudo unit of structure of Tussah silk, viewed along the fiber axis.

for all of the main-chain atoms and for the β-carbon atoms of the side chains are listed in Table 3. The parameters are consistent with a planar peptide group and linear hydrogen bonds; they lead to the bond distances and bond angles listed in Table 4, where the accepted values (Corey & Pauling, 1953) are also listed for comparison. The shortest distance between non-bonded atoms in neighboring sheets is 3·9 Å.

The symmetry of the pseudo structure is that of the space group D_2^4-$P2_12_12_1$; there are eight amino-acid

Table 3. *Atomic parameters for the pseudo unit of structure of Tussah silk*

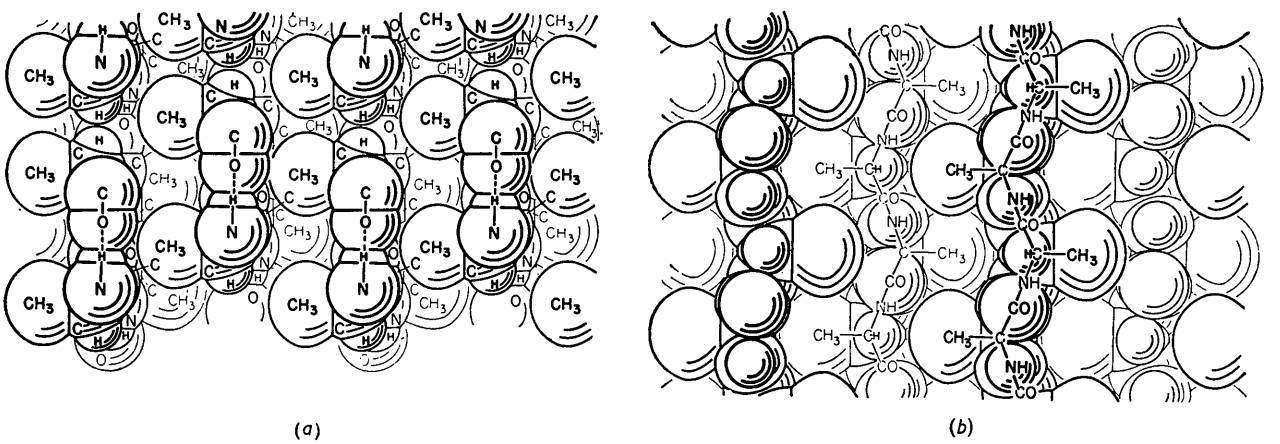
Space group: $P2_12_12_1$.
Equivalent positions: (x, y, z); $(\frac{1}{2}-x, \bar{y}, \frac{1}{2}+z)$; $(\bar{x}, \frac{1}{2}+y, \frac{1}{2}-z)$; $(\frac{1}{2}+x, \frac{1}{2}-y, \bar{z})$.
$a_0 = 9{\cdot}44$, $b_0 = 6{\cdot}95$, $c_0 = 10{\cdot}60$ Å.

	Residue I					Residue II				
Atom	C′	O	N	C	β-C	C′	O	N	C	β-C
x	0·328	0·197	0·404	0·340	0·340	0·422	0·553	0·346	0·410	0·410
y	0·676	0·676	0·824	0·002	0·002	0·176	0·176	0·324	0·502	0·502
z	0·514	0·514	0·478	0·434	0·291	0·486	0·486	0·522	0·566	0·709

Table 4. *Interatomic distances and bond angles for the pseudo structure*

Distances	Calculated from parameters in Table 3	Accepted values*	Angle	Calculated from parameters in Table 3	Accepted values*
C′=O	1·24 Å	1·24 Å	O=C′−N	123°	123°
C′−N	1·31	1·32	O=C′−C	120	121
N−C	1·45	1·47	C−C′−N	116	114
C−C′	1·54	1·53	C′−N−C	122	123
C−βC	1·52	1·54	C′−N · · · O	122	123
O · · · N	2·77	2·79	C−N · · · O	115	114
			N−C−C′	110	110
			N−C−βC	109	109½
			C′−C−βC	111	109½

* Corey & Pauling, 1953.



(*a*) (*b*)

Fig. 3. A packing drawing of the pseudo structure of Tussah silk (*a*) viewed along the fiber axis, (*b*) viewed perpendicular to the fiber axis and parallel to the plane of the pleated sheets.

residues within the pseudo unit cell, and hence two (I and II in Table 3) within each asymmetric unit. They are successive residues of a single polypeptide chain. Thus, the space group differs from that derived for the pseudo unit cell of *Bombyx mori*—$P2_1$. The difference is due to the method of packing of adjacent pleated sheets; in *Bombyx mori* adjacent sheets are separated by distances alternately 3·5 and 5·7 Å, whereas in Tussah silk the sheets are spaced at equal intervals (5·3 Å).

The parameters of Table 3 were used in the calculation of structure factors for the equatorial reflections and those occurring on the first three layer lines. Atomic form factors of McWeeny (1951) were used; no temperature factor was applied. The resulting values of F^2 are listed in Table 5. The agreement with the observed intensities is seen to be quite satisfactory; it could be improved by the application of an anisotropic temperature factor to take account of the apparent disorder in the c direction which is manifested by the diffuseness of the $00l$ reflections. It should be pointed out that the calculations included one β-carbon atom for each residue of the structure, whereas the results of chemical analysis of Tussah silk (Schroeder & Kay, 1955) indicate the presence of about 27% glycine, and hence approximately one-quarter of the β-carbon atoms are in fact replaced by hydrogen atoms.

Discussion of the structure

Packing drawings of the pseudo structure of Tussah silk fibroin are shown in Fig. 3. Within each pleated

Table 5. *Observed intensities for Tussah silk compared with values of F^2 calculated for the pseudo unit of structure*

		$k = 0$		$k = 1$		$k = 2$		$k = 3$	
$h\,l$	d_{h0l}	I_o	F_c^2	I_o	F_c^2	I_o	F_c^2	I_o	F_c^2
0 0	—	—	—	—	0		5	—	0
0 1	10·60 Å	—	0	—	0	*ms*	0		0
1 0	9·44	—	0	—	15		193	*m*	132
1 1	7·05	—	1	—	16	—	10		106
0 2	5·30	*vvvs*	1248	*vs*	239	*s*	390	—	7
2 0	4·72	—	0	—	0	—	0	*ms*	0
1 2	4·62	—	0	—	33	—	10		69
2 1	4·31	*vvs*	444	*vs*	260	—	12	—	45
0 3	3·53	—	0	—	0	—	0	—	0
2 2	3·53	—	0	—	0	—	0	—	0
1 3	3·31	—	8	—	28	—	6		55
3 0	3·15	—	0	—	18	—	0	**mw*	10
3 1	3·02	—	18	—	3	*w*	79		48
2 3	2·83	—	24	*w*	89	—	36		3
3 2	2·71	—	7	—	14	*vvw*	71	—	12
0 4	2·65	*s*	900	—	37		120	—	57
1 4	2·55	—	13	—	13	—	7		50
4 0	2·36	*mw*	233	*mw*	87	*w*	16	**vw*	1
3 3	2·35		1		19		47		7
2 4	2·31	—	0	—	0	—	0		0
4 1	2·30	—	0	—	0	—	0	—	0
4 2	2·16	—	26	*w*	102	—	17	—	8
0 5	2·12	—	0		0	—	0	—	0
1 5	2·07	—	8	—	9	—	12	—	38
3 4	2·03	—	3	—	13	—	34	—	15
4 3	1·96	—	0	*vvw*	0	—	0		0
2 5	1·93	—	47		100	—	4	*vvw*	4
5 0	1·89	—	0	—	27	—	37		72
5 1	1·86	—	18	—	5	—	22	—	35
5 2	1·78	—	9		4		20	—	4
0 6	1·77	—	161		182		164	—	7
3 5	1·76	—	0	*vvw*	13	*vvw*	34	—	12
4 4	1·76	—	33		44		8	—	4
1 6	1·74	—	7	—	11		13	—	19
5 3	1·67	—	0	—	6	—	4	—	2
2 6	1·65	—	0	—	0	—	0	—	0
6 0	1·57	*w*	0	—	0	*vvw*	0	*w*	0
6 1	1·56		103	—	8		13		1

* These two reflections appear to be double.

The brackets around the F_c^2 values include all reflections having scattering angles within the range imposed by the uncertainties of the measurements of the observed maxima.

sheet, adjacent polypeptide chains are held together in an antiparallel sense by linear hydrogen bonds (Pauling & Corey, 1953). Adjacent pleated sheets are separated by a distance of 5·3 Å ($= \frac{1}{2}c_0$), the space between sheets being occupied by the side-chain atoms of the various amino-acid residues. In Fig. 3, all of the side-chains are represented as equivalent; their size as shown is approximately that expected for the methyl groups of alanine residues. The side chains of adjacent pleated sheets interlock in a highly efficient manner, a side chain of one sheet being surrounded by four side chains of the next sheet. Furthermore, as can be seen in Fig. 3(*b*), each side chain falls in the concavity created by the 'pleating' of the adjacent sheet.

We can now see how the differences in the structures of *Bombyx mori* and Tussah silk fibroins are related to the striking differences in their chemical compositions. The principal difference between the pseudo structures of the two silks is in the method of packing of the pleated sheets. The predominant feature of the structure of *Bombyx mori* is the alternation of intersheet distances between the values 3·5 and 5·7 Å; these distances correspond to back-to-back and front-to-front packing, respectively, between pleated sheets having the methyl-group side chains of alanine (or serine) residues protruding from the back side. Such pleated sheets can be formed from the polypeptide chains of *Bombyx mori* in which the residues are alternately glycine and alanine (or serine); the sequence *–G–X–G–X–G–* (*G* = glycine, *X* = alanine or serine) occurs frequently in this silk. In Tussah silk, however, adjacent pleated sheets are spaced regularly at a distance of 5·3 Å; thus, both sides of the pleated sheets appear to be structurally equivalent and the X-ray data give no information concerning the sequence of residues.

These structural differences can be readily explained in light of the chemical compositions of the two silk

fibroins (see Table 1). In *Bombyx mori*, glycine accounts for about 44% of the amino-acid residues, and alanine and serine together make up an additional 42%. Thus, a sequence of the type *–G–X–G–X–G–* will account for about 85% of the residues. In Tussah silk, however, the amount of glycine is quite insufficient to allow such a sequence to predominate in the structure. Accordingly, the pleated sheets in Tussah silk cannot arrange themselves in the manner found in *Bombyx mori*; instead, they adopt another simple structure which is particularly appropriate in view of the high alanine content.

It should be emphasized that, as in the case of *Bombyx mori*, the structure we have derived for Tussah silk must be regarded as only a pseudo structure. On the basis of the proposed unit cell, containing eight amino-acid residues, and an assumed density of about 1·35 g.cm.$^{-3}$, the average residue weight is calculated to be about 71. This value is exactly the weight of an alanine residue, but is considerably smaller than the value 83·5 calculated for Tussah silk from the data of Schroeder & Kay (1955). Furthermore, the distance between adjacent pleated sheets, 5·3 Å, is insufficient to accommodate the side chains of the larger amino-acid residues, such as tyrosine. There seems to be no valid reason for presuming that the crystalline portion of Tussah silk contains only the smaller amino-acid residues glycine, alanine and serine; rather, it is probable that there are regions in the structure where adjacent pleated sheets are separated by distances greater than 5·3 Å.

A note on the structure of β-poly-L-alanine

Bamford, Brown, Elliott, Hanby & Trotter (1954) have reported that the X-ray diffraction pattern of the β (stretched) form of poly-L-alanine is almost identical to that of Tussah silk; they have proposed a unit cell for polyalanine which, except for a halving of the *a* axis, has dimensions in good agreement with our pseudo unit cell of Tussah silk. In view of the large amount of alanine in Tussah silk and the efficiency of packing of alanine residues in the pseudo structure, it seems likely that the structure of β-poly-L-alanine is very closely related to the pseudo structure which we have derived for Tussah silk fibroin. As pointed out previously in this paper, the average residue weight calculated for the pseudo structure of Tussah silk, assuming a density of 1·35 g.cm.$^{-3}$, is 71, the weight of an alanine residue.

Bamford *et al.* report two significant differences between the diffraction patterns of polyalanine and Tussah silk. First, they find (Bamford *et al.*, 1953) that the fiber-axis identity distance in poly-L-alanine is slightly shorter than in Tussah silk fibroin; this shortening, which may connote a slightly greater twist in the polypeptide chains, is too small to require significant revision of the atomic positional parameters listed in Table 3. Second, they report (Bamford *et al.*, 1954) that the intensity of the 5·3 Å equatorial reflection is distinctly lower in Tussah silk than in poly-L-alanine. This reflection, which has been indexed as 002 on the basis of our pseudo unit cell, is the first order of the spacing between adjacent pleated sheets. Thus, any distortions in the packing of the sheets, as might be required by the large amino-acid residues in Tussah silk, would be expected to lower the intensity of this reflection; no such distortions would be expected in poly-L-alanine.

In view of the experimental evidence, it is difficult to escape the conclusion that the structure of β-poly-L-alanine is essentially that of the pseudo unit of Tussah silk formulated in Table 3.

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