ANALYSIS OF THE X-RAY DIFFRACTION PATTERN OF HELICAL STRUCTURES

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ABSTRACT

The paper deals with the theory of the diffraction pattern of helical structures having the number of units per turn (n) neither integral nor rational. The conventional treatment suffers from the defect that the repeat spacing along the axis of the helix is taken as the standard of reference, and this does not exist, being infinite, when n is irrational. The difficulty is got over in this paper by focussing attention on the 'unit height' (h = resolved component of a unit along the axis) and 'unit twist' (t = fraction of a complete rotation for one unit, $= 1/n$), which vary continuously irrespective of n being rational or irrational. Explicit formulæ are obtained in terms of their Bessel indices for the observed layer line-spacings which turn out to be very simply related to the reciprocals of the unit height and the pitch of the helix. A technique of analysing the observed diffraction pattern for the elements of the helical structure is also given, with examples. The case of a coiled-coil is seen to have the same general features as the simple coil, the layer line-spacing being now related to two pitches, namely, those of the major and the minor helices, and the unit height. The relationship of the diffraction pattern of a helix in its uncoiled and its coiled-coil form is also found to be rather simple, being similar to the multiplet splitting produced by a magnetic field in spectral lines.

1. THEORY FOR A SIMPLE HELIX WITH A NON-INTEGRAL SCREW AXIS

IT is well known that the characteristic feature of the X-ray diffraction pattern of a fibre containing helical chains, with the number of links (units) per turn, n, non-integral, is the occurrence of strong layer lines whose layer-spacings are not all in the ratio of 1 : 2 : 3, etc., as is found in the rotating crystal diagram of an ordinary crystal. An explanation of this was given in terms of the Fourier transform of a helical structure by Cochran, Crick and Vand (1952). Taking in particular the case of M point atoms arranged at regular intervals in N turns of a helix of radius r and pitch P, so that the

repeat spacing along the axis is $c = NP$, the intensity distribution in reciprocal (Fourier) space (R, ψ, Z) on the layer line l with $Z = l/c$ essentially depends on the value of the Bessel function $J_q(2\pi Rr)$, where q is an integer which satisfies the equation

$$Nq + Mm = l \qquad (q,\ m,\ l \text{ integers}). \tag{1}$$

Now denote the projected distance between two successive atoms along the axis of the helix by $h\,(= c/M)$, which may be called the 'unit height', and the fraction of a full rotation corresponding to one such unit by $t\,(= N/M)$, which may similarly be called the 'unit twist'. Clearly the unit twist is the reciprocal of the number of units per turn, *i.e.*,

$$t = \frac{1}{n}. \tag{2}$$

Since only $J_0(x)$ has a finite value for $x = 0$, all other $J_q(x)$ being zero for $x = 0$, and since the first maximum of $J_q(x)$ occurs for larger and larger values of x and is relatively lesser in height as q increases, it follows that the prominent layer lines are those for which q is a small integer and of these only those with $q = 0$ contain a meridional reflection. Thus, restricting ourselves to layer lines with spacings between c and $c/M = h$, *i.e.*, $l = 1$ to M, the principal layer lines are seen to be as below*:

For $q = 0$,	$l = M$;	$Z = M/c$;
For $q = 1$,	$l = N,\ M - N$;	$Z = N/c,\ (M - N)/c$;
For $q = 2$,	$l = 2N,\ M - 2N$;	$Z = 2N/c,\ (M - 2N)/c$.

Now, suppose that the helix undergoes a small deformation, so that the unit twist t, which was equal to N/M, increases very slightly (say). It is obvious that the repeat spacing c would completely change, *e.g.*, taking the α-helix with $h = 1{\cdot}5$ A, $n = 3{\cdot}6$, which corresponds to $N = 5$, $M = 18$, $c = 27$ A, if n increases to $3{\cdot}625$, then $N = 8$, $M = 29$, $c = 43{\cdot}5$ A, while if n increases to $3{\cdot}61$, then $N = 100$, $M = 361$, $c = 541{\cdot}5$ A. Thus, the repeat spacing c undergoes wild fluctuations as the number of units per turn (n) or t is changed even by a minute amount—actually $c \to \infty$ if n is irrational. However, it stands to reason from physical grounds that the diffraction pattern would only undergo an infinitesimal change for such an infinitesimal change in n or t. Actually, that this is so can be verified by

* These conclusions continue to hold even when there are a number of atoms in the repeating unit, provided they all follow the same helical pattern, as is the case in a helical structure, which is fully specified by the two parameters h and t (or n).

fully working out the pattern according to the above theory for various rational approximations to a given number. This point has been recognised by Cochran *et al.* (1952), but they have not considered the general case. It would, therefore, be worthwhile reformulating the theory in such a manner that no such rational approximation is needed. As will be shown below, it turns out that what is relevant to the specification of a helical structure are the parameters unit height (h) and the unit twist (t). The repeat spacing c need not enter the picture at all in the formulation. It is obvious that, from the stereochemical point of view also, the only data that are specified for a helical chain is the relationship between one unit and the next, which is precisely the same between that and the next and so on. The repeat spacing c is never specified as such.†

Thus, since c is unspecified, the only reciprocal spacing which can be used to describe the pattern is the reciprocal of the unit height h, *i.e.*, $1/h = Z_0$ (say). This is in fact the spacing of the meridional reflection. Therefore, let $Z = \eta Z_0$, instead of l. $(1/c)$ as before. Then, in Eqn. (1) $l = \eta c/h = \eta M$, so that we have

$$Nq + Mn_i = \eta M,$$

or dividing by M throughout and remembering that $N/M = t$, we have

$$tq + m = \eta, \qquad Z = \frac{\eta}{h} = \eta Z_0. \tag{3}$$

The layer lines are now given by the number η, which is not an integer (and which is not even necessarily a rational number). The prominent layers correspond to $q = 0, 1, 2, \ldots\ldots$ in decreasing order of prominence. Consequently, it would be more useful to use the Bessel index q to denote the layer lines in the diffraction pattern of a helix, and the corresponding $\eta_q = (tq + m)$ gives its location.

In order to get a clearer picture, let us restrict ourselves to layers with $|Z| < 1/h$, *i.e.*, those occurring within the first meridional reflection. Taking $n = 3{\cdot}6$ as for the α-helix, $t = 0{\cdot}278$ and the layers with Bessel indices $|q| = 0, 1, 2, 3, 4$ occur at $Z = Z_0$, $Z = 0{\cdot}278Z_0$ and $0{\cdot}722Z_0$, $Z = 0{\cdot}556$ and $0{\cdot}444Z_0$, $Z = 0{\cdot}834$ and $0{\cdot}166Z_0$, $Z = 0{\cdot}112$ and $0{\cdot}888Z_0$.

† It has been possible to work out specific formulæ, which give (*a*) the orientation of the helical axis, (*b*) the unit height and (*c*) the unit twist (t) when the stereochemical relations between successive units are given, but this will be reported separately.

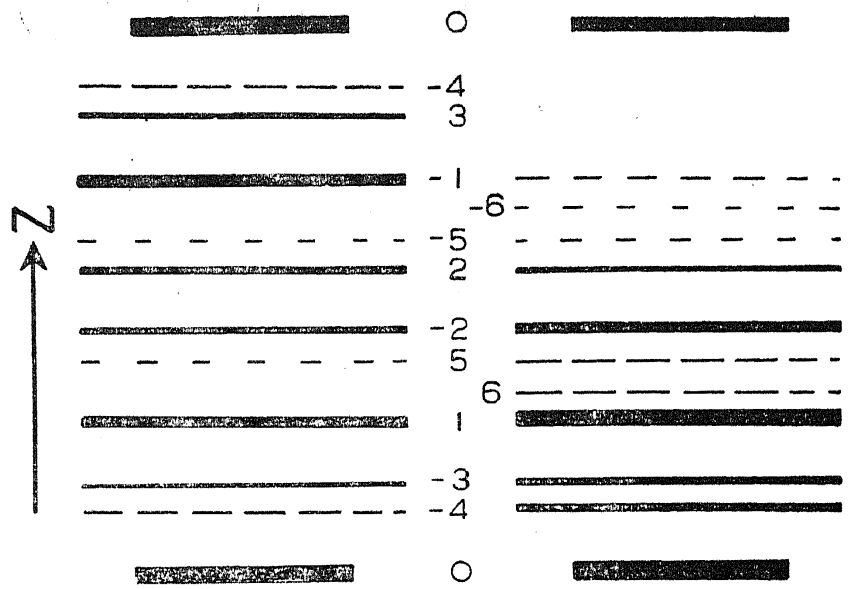

FIG. 1. (*a*) Schematic diagram, showing layer lines of a simple helix with $n = 3{\cdot}6$. The thickness of each layer roughly represents its intensity. (*b*) Relative intensities of the layers actually observed with poly-L-alanine. Note the occurrence of layers even with large q (up to 6) at low angles (small Z), but the relative absence of layers at larger values of Z.

These are schematically shown in Fig. 1 and the general formula may be written in the form:

$$Z_q = [[qt]]\, Z_0 \quad \text{and} \quad Z_q' = (1 - [[qt]])\, Z_0, \tag{4}$$

where $[[x]]$ stands for the fractional part of x. Those with $q = 0$ are meridional, while the others are non-meridional. In terms of the X-ray crystallographic $\zeta = \lambda Z$, the ζ-values of the prominent layer lines can be deduced from Eqn. (4) to occur at

$$\zeta = [[qt]]\frac{\lambda}{h} \quad \text{and} \quad \zeta = (1 - [[qt]])\frac{\lambda}{h}. \tag{5}$$

It will be noticed that these very simple formulæ contain no reference at all to the repeat spacing c, but require only the specification of h, the projected height of one unit along the axis of the helix and n, the number of units per turn. The formulæ are equally valid when n is integral or is the ratio of (simple) integers. Since, in the limit when n tends to an irrational number, there is no quantity in the formulæ which fluctuates indefinitely, the formulæ are also true in the limit when n is irrational. Thus, they can be taken to be valid quite generally. Then, the intensity distribution in reciprocal space on a layer of Bessel index q is given by the formula of Cochran *et al.*, *viz.*,

$$F(R, \psi, Z) = \sum_j \sum_q f_j J_q (2\pi R r_j) \exp i \left[q\left(-\psi + \phi_j + \frac{\pi}{2}\right) + 2\pi Z z_j \right] \quad (6)$$

where (r_j, ϕ_j, z_j) is the position of a j-th type atom and f_j is its scattering factor, and Z is given by Eqn. (3).

2. Analysis of Layer Lines by the New Formula

The new form of the diffraction formula for a helical structure given by Eqn. (3) will be found to be very useful in practice as will be evident from some examples to be given below. Thus, in interpreting a helix diffraction pattern, the first thing to look for should be the truly meridional reflections. Owing to disorder, some non-meridional reflections would occur as arcs bridging the meridian, but these can in many cases be resolved by taking inclined fibre photographs. So also, the truly meridional reflection can be brought out by such a photograph, as in the case of the 1·5 A of the alpha helix. Having thus obtained h and hence ζ_0 (or Z_0), the next step should be to look for the other prominent layer lines with $\zeta < \zeta_0$. If two such can be found, say ζ, ζ' such that

$$\zeta + \zeta' = \zeta_0, \quad (7)$$

then it is clear from Eqn. (5) that

$$\zeta, \zeta' = (qt)\,\zeta \quad \text{and} \quad (1 - qt)\,\zeta_0, \quad (8)$$

most probably with $q = 1$, so that ζ/ζ_0 gives at once the unit twist t, and n, the number of units per turn of the helix. Sometimes q may not be equal to one, but 2 for the most prominent layer lines owing to the fact that the Fourier transform of the unit itself is weak near the meridian corresponding to the layers with $q = 1$. In any case, further confirmation of the assignment can be obtained by comparing the ζ-values of the other observed layer lines with the values calculated from Eqn. (5).

Thus, it is seen that the analysis of the X-ray diffraction pattern of a helix for its elements h and t (or n) is quite straightforward, so long as the layer lines are clear and the meridional and non-meridional reflections can be clearly distinguished. We shall illustrate the procedure with reference to the data on the α-form of poly-γ-methyl-L-glutamate (Bamford *et al.*, 1956). The mean values of ζ for the observed layer lines are 0·109, 0·173, 0·2865, 0·461 and 1·028, the last of which is meridional. The strongest layer is the one with $\zeta = 0{\cdot}2865$.

Obviously $\zeta_0 = 1{\cdot}028$, giving $h = 1{\cdot}50$ A. A more accurate value of $\zeta_0 = 1{\cdot}034$ may be deduced from the later data of Brown and Trotter (1956) which is also in agreement with the measurement $h = 1{\cdot}491$ A of Yakel (1953). No two of the ζ's satisfy an equation of the form (7). However,

$$2 \times 0{\cdot}2865 + 0{\cdot}461 = 1{\cdot}034$$

and

$$3 \times 0{\cdot}2865 + 0{\cdot}173 = 1{\cdot}0325$$

and the sum in either case is close to $\zeta_0 = 1{\cdot}034$. From the general theory, these equations are consistent with the assignment

$$0{\cdot}2865 = t\zeta_0 \qquad (q = 1)$$
$$0{\cdot}461 = (1 - 2t)\,\zeta_0 \qquad (q = -2)$$
$$0{\cdot}173 = (1 - 3t)\,\zeta_0 \qquad (q = -3)$$

The mean value of $n\,(= 1/t)$ obtained from these assignments is $3{\cdot}58$. This value leads to $\zeta_4 = (4t - 1)\,\zeta_0 = 0{\cdot}106$, which is close to the observed value of $0{\cdot}109$.

Thus, our analysis shows that the observed pattern is consistent with a structure with $h = 1{\cdot}491$ A and $n = 3{\cdot}58$. Actually Bamford *et al.* have tried to index their observations on the basis of a cell with M = 29, N = 8 giving $n = 3{\cdot}625$ and M = 69, N = 19, *i.e.*, $n = 3{\cdot}632$ in addition to the simple $n = 3{\cdot}6$ helix with M = 18, N = 5. If $\zeta_0 = 1{\cdot}028$ is used, the mean value of n obtained is $3{\cdot}61$ (variation $3{\cdot}58$ to $3{\cdot}64$), which is close to the rational approximation attempted. These facts suggest that the rational approximations are not particularly relevant, unless they are dictated by some special considerations such as symmetry.

A similar analysis may be made of the pattern of α-poly-L-alanine (Brown and Trotter, 1956; Bamford *et al.*, 1956). Brown and Trotter have given a table of observed ζ's of the various layer lines conveniently as reciprocal spacings Z in A^{-1}. We shall designate the layers by A, B, C....K whose Z-values are listed in Table I. The last one K with $Z_K = Z_0 = 0{\cdot}668_6$ is the only meridional reflection, giving $h = 1{\cdot}495$ A.

Of these, $Z_C + Z_J = 0{\cdot}665 \simeq 0{\cdot}668_6$ and C is a prominent layer line, so that we may identify these with $q = 1$, giving $Z_C = tZ_0$ and $Z_J = (1 - t)\,Z_0$. Now, from Eqn. (3) it follows that

$$\frac{Z}{Z_0} = \eta = m \pm qt. \qquad (9)$$

TABLE I

List of observed layer lines and their indexing by Bessel indices q *for poly-L-alanine. Note that layer lines with* q = 3, 4 *at* 0·555 *and* 0·597 *are absent while those with* q = 5, 6 *actually occur*

Layer line	Z	|q|	Z/Z_0 (from theory)	$Z_1 = tZ_0$ (from obsn.)
A	0·0713	4	$4t-1$	0·1850
B	0·114	3	$1-3t$	0·1849
C	0·185	1	t	0·1850
D	0·233	6	$2-6t$	0·1840
E	0·257	5	$5t-1$	0·1851
F	0·298	2	$1-2t$	0·1858
G	0·367	2	$2t$	0·1835
H	0·411	5	$2-5t$	0·1852
I	0·433	6	$6t-1$	0·1836
J	0·480	1	$1-t$	0·1886*
K	0·6686	0	1	..
			Mean ..	0·185 ± 0·001

* Omitted in obtaining the average and standard error.

From this, it is obvious that a number of observed Z-values must differ by multiples of $Z_1 (= tZ_0)$. It will be seen that this is in fact true. Thus,

$$Z_E - Z_A = 0{\cdot}185$$
$$Z_F - Z_B = 0{\cdot}184$$
$$Z_G - Z_C = 0{\cdot}182$$
$$Z_H - Z_D = 0{\cdot}178$$
$$Z_I - Z_E = 0{\cdot}176$$
$$Z_J - Z_F = 0{\cdot}182$$
$$Z_K - Z_J = 0{\cdot}188_6$$

This clearly establishes that our identification of $Z_1 = 0{\cdot}185$ by $q = 1$ is correct. A further confirmation of this comes from the fact that $Z_F + Z_C = 0{\cdot}665 \simeq Z_K$ and the relation $Z_F \simeq 2Z_C$ holds. The indexing of all the observed layers is, thereafter, straightforward. The values of q are given in the third column and the value of Z/Z_0 from theory in the fourth column.

Using the data from the fourth column, the value of $Z_1 (= tZ_0)$ is deduced from each of the measurements and they are given in column 5. It will be seen that all of them are very nearly a constant except the one deduced from Z_J which has a larger error. This layer is very weak in the diagram and so this value may be omitted in finding the mean which comes out to be $0{\cdot}185 \pm 0{\cdot}001$, where the $\pm$ gives the standard error. Taking the value of Z_0 to have a relative error of $\pm 0{\cdot}2\%$, as given by Brown and Trotter, we find that

$$n = 3{\cdot}615 \pm 0{\cdot}02.$$

It is interesting that the mean value is identical with that assumed by Brown and Trotter, namely, $47/13 = 3{\cdot}615$. However, their quoted probable error $\pm 0{\cdot}003$ is far too small. It is not clear how they have deduced their probable error, but their own data giving $\zeta_{obs.}$ and $\zeta_{cal.}$ in their Table I (their ζ is our Z) show a percentage deviation of the order of 1% (*e.g.*, 0·233–0·228, 0·433–0·441, 0·480–0·484, etc.) which would be more in accord with our standard error (0·6%) than theirs (<0·1%). This shows that the experimental data cannot prove that a chosen rational approximation is the correct one.

Thus, it will be seen that the analysis of the diffraction pattern of a simple helix can be made into a simple routine. This is so even if the truly meridional reflection does not record, for Z_0 can be deduced from the fact that pairs of Z-values will have their sums equal. In the present case, we have several such, *e.g.*,

$$\begin{aligned} Z_J + Z_C &= 0{\cdot}665 \\ Z_I + Z_D &= 0{\cdot}666 \\ Z_H + Z_E &= 0{\cdot}668 \\ Z_G + Z_F &= 0{\cdot}665 \\ \text{Mean} &= 0{\cdot}666 \end{aligned}$$

It will be noticed that the mean is nearly equal to the directly measured value of Z_0 (namely 0·6686) which is highly satisfactory. Modifications of these methods could, of course, be worked out to suit particular cases.

So also, it is seen that, in the ordinary way, it is not possible to prove that there is a definite repeat spacing c in which the helix makes an integral number of turns. Actually, in such structures, which are not stablilised by rigid bonds connecting one helix with another, small variations are likely to occur, and the interesting fact that has come out of the present study is that such variations only insignificantly affect the diffraction patterns. The layer lines may be slightly shifted about (not all in the same direction), but the strong ones would continue to be strong and the weak ones weak.

3. THEORY FOR A COILED-COIL

In the above discussion, we have been dealing only with simple helices. When there is a further coiling of these, leading to superhelical configurations, the nature of the diffraction pattern changes somewhat. In order to get the picture clearly, we shall write the formula of Crick (1953) connecting the various Bessel functions orders in a form more akin to our equations (1) and (8). His equation is, with a small change in notation (N_1' instead of N_1),

$$N_0 p + (N_1' - N_0)\, q + N_1' s + (N_1' + N_0)\, d + Mm = l,$$

with p, q, s, d, m, l integers (10)

for a layer line with $Z = l/c$, c being the repeat spacing which contains M units, N_0 turns of the major helix and N_1' turns of the minor helix. Let $n_0 = M/N_0$ be the number of units per turn of major helix, $n_1' = M/N_1'$, the number of units per turn of the minor helix in a rotating frame of reference following the major helix. As in the case of the simple helix, write $Z = \eta \cdot (1/h)$, so that $l = \eta M$ and let $t_0 = 1/n_0$, $t_1' = 1/n_1'$. Then Eqn. (10) becomes

$$t_0 p + (t_1' - t_0)\, q + t_1' s + (t_1' + t_0)\, d + m = \eta \qquad (11\,a)$$

$$Z = \eta Z_0,\ Z_0 = \frac{1}{h}. \qquad (11\,b)$$

Thus, the repeat spacing c has disappeared and the layer is now specified by the number η (not necessarily even rational) which corresponds to the Bessel indices (p, q, s, d). The intensity distribution in this layer continues to be given by Crick's Eqn. (13) for a coiled-coil with one atom per unit having unit scattering factor (*see* his paper for details of notation):

$$F(R, \psi, Z) = \sum_p \sum_q \sum_s \sum_d J_p(2\pi R r_0)\, J_q(2\pi R \bar{r}_1) \,.\, J_s(2\pi Z r_1 \sin\alpha)\, J_d(2\pi R \Delta) \times \exp i \left[p\left(\psi - \phi_0 + \frac{\pi}{2}\right) + q\left(-\psi + \phi_1 + \frac{\pi}{2}\right) + s(-\phi_1 + \pi) + d\left(\psi + \phi_1 + \frac{\pi}{2}\right) - m\phi_M + 2\pi Z z_0 \right]. \quad (12)$$

The most prominent layer lines correspond to small values of p and q. The index $s = 0$ for all layer lines with $Z \ll Z_0$ but $s = 1$ may occur for $Z \sim Z_0$. The index d must be put zero in most cases.

Before working out the consequences of Eqn. (11) under these conditions, it is worthwhile examining the relation between the transform of a coiled-coil and of the simple coil from which it has been obtained. Now in Crick's paper, N_1' is the number of rotations made by the minor helix in a rotating frame which rotates along with the major helix. (Crick calls this 'the number of rotations made by the minor helix *in its own frame of reference*', which appears to be not a happy wording, although its connotation is quite clear in his paper.) Now consider the number of turns N_1 made by the minor helix in space when the major helix makes N_0 turns. Under the conditions adopted in Crick's paper (left-handed minor helix, right-handed major helix, *i.e.*, the senses are opposite), it will make a smaller number of turns than N_1', actually $N_1 = N_1' - N_0$. The significance of N_1 is best visualised by considering the case when the radius of the major helix shrinks to zero. There is no coiled-coiling in such a case and N_1 is then the number of turns made by the minor helix in the repeat c. The number N_1 thus specifies the undistorted minor helix (and not N_1') and consequently it is used in all the formulæ derived below.

Thus, let N_1 be the number of turns of the minor helix in the repeat c. The associated number $n_1 = M/N_1$ is the number of units per turn of the minor helix and its reciprocal $t_1 = 1/n_1$ is its unit twist. In terms of these, Eqns. (10) and (11) become

$$N_0 p + N_1 q + (N_1 + N_0)\, s + (N_1 + 2N_0)\, d + Mm = l \quad (13)$$

$$t_0 p + t_1 q + (t_1 + t_0)\, s + m = \eta \quad (14)$$

where d is put zero in Eqn. (14). Eqn. (12) continues to be valid with this notation also.

We shall now consider the consequences of these equations.

(*a*) $s = 0$.

As already mentioned, $J_s(2\pi Zr_1 \sin\alpha)$ does not have appreciable values for small Z, except for $s = 0$. In such a case, we get

$$t_0 p + t_1 q + m = \eta, \quad Z = \eta Z_0 \tag{15}$$

which may be compared with Eqn. (3) where $t = t_1$. In general t_0, the unit twist of the major helix, will be much less than t_1 that of the minor helix so that $t_0 p \ll t_1 q$. Thus the effect of coiled-coiling is to split up each of the layers given by the simple helix into a series of closely spaced layers, becoming weaker on either side of the original one for the simple coil. This follows from the fact that $J_p(2\pi R r_0)$ and $J_q(2\pi R \bar{r}_1)$ occur multiplied together. The situation is shown schematically in Fig. 2 for two cases (*a*) $t_0 \ll t_1$ and (*b*) $t_0 \sim t_1$. There is a close analogy to the multiplet structure of spectral lines, which might have struck the reader even in the case of the simple helix diffraction pattern (*e.g.*, the occurrence of constant differences, constant sums, etc.). The multiplets (p different) may be close together or well separated depending on t_0 being small compared to t_1 or not.

Thus, it is seen that there is no meaning in asking whether supercoiling exists or not, but only whether it is appreciable or not, for when the pitch of the major helix is very large, we would only find a slight broadening of the observed layer lines.

(*b*) $s \neq 0$.

Since $(t_0 p + t_1 q)$ contains also all the values occurring in the sum $t_0 p + t_1 q + (t_0 + t_1) s + (t_0 + 2t_1) d$, no layer lines can occur at positions other than those given by Eqn. (15) and sketched in Fig. 2. On the other hand, the functions J_s and perhaps also J_d with $s, d \neq 0$ may also contribute to some of the reflections. Thus, their intensity might be quite prominent.

Important examples of this type are the possible layer lines near Z_0 ($\eta \simeq 1$) for which $J_1(2\pi Zr_1 \sin\alpha)$ with $s = 1$ has an appreciable value. In such a case, for $q = 0$, $p = -1$, -2, etc., there occur layer lines at

$$Z = Z_0, \; Z_0(1 - t_0), \; Z_0(1 - 2t_0), \; \text{etc.},$$

i.e., a series of layer lines with constant differences corresponding to $t_0 Z_0$. Such layer lines cannot occur for a simple helix, and since $q = 0$, they will occur very near the meridian.

In fact the simplest way of finding the value of t_0 seems to be to look for such layers close to $Z = 0$ and $Z = Z_0$.

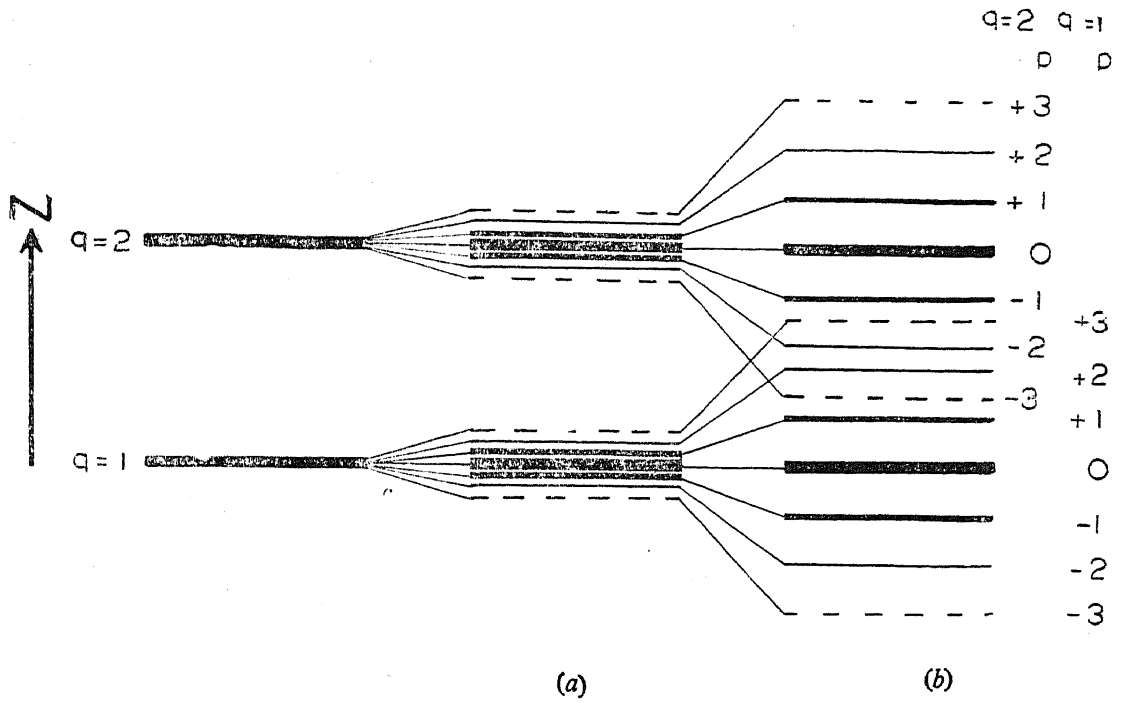

FIG. 2. Splitting of layer lines due to coiled-coiling.

Thus, the main point of interest is that the diffraction pattern of a coiled-coil is not frightfully complicated, in spite of the fact that Eqn. (13) giving the Bessel indices contains four different parameters. In terms of our analysis, it is seen that the layer lines have only two types of periodicities:

(*a*) $t_0 Z_0$ due to the major helix

(*b*) $t_1 Z_0$ due to the minor helix

and the actual layer line spacings are given by Eqn. (15). Consequently, if the Z-values of the layer lines are accurately measured, they must be fitted to a formula of the type

$$Z = mZ_0 + pE_0 + qE_1 \quad (m,\ p,\ q \text{ integers}). \tag{16}$$

Of these, the layer line with $Z = Z_0$ has intensity on the meridian, those with $Z = Z_0 \pm pE_0$ have intensity close to the meridian, while the others are definitely non-meridional. Having found Z_0, E_0, E_1, the elements of the coiled-coil are given by

$$h = \frac{1}{Z_0}, \quad t_0 = \frac{E_0}{Z_0}, \quad t_1 = \frac{E_1}{Z_0}. \tag{17}$$

In view of the above results, the nature of the diffraction pattern when there is a still further coiling is easy to work out. If n is the number of units per turn in the primitive coil, n' the corresponding number for the first superhelix and n'' for the second superhelix, then the layer lines which occur are at

$$Z = \eta Z_0 \qquad (18\,a)$$

where

$$\eta = t''u + t'p + tq + m \ (u, p, q, m \text{ integers}) \qquad (18\,b)$$

and

$$t = \frac{1}{n}, \quad t' = \frac{1}{n'}, \quad t'' = \frac{1}{n''} \qquad (18\,c)$$

analogous to Eqn. (15). Obviously, layers with u, p, q small will be the strong ones.

4. CONCLUSION

If one examines Eqns. (3), (15) and (19) respectively for a simple helix, a coiled-coil and a coiled-coiled-coil, it will be seen that they are all of the same essential form. The strong layer lines occur at Z-values which, when expressed as a fraction η of the fundamental reciprocal spacing $Z_0 = 1/h$, have a direct relation to the unit twists of the various helices. In the simple helix, the various η's are just multiples of the unit twist (*cf.* $\eta = tq + m$, with q, m integral). With further coiling, the periodicities of the other (major) helices show up as a fine structure modulating the diffraction pattern of the simple helix.

In other words, if the observed data are plotted on the η-scale, *i.e.*, dividing the observed reciprocal spacing of the unit height, then they would exhibit one, two or more periodicities, according to the number of coilings in the strucure. The problem is then only of finding this periodicity, taking into account the fact that some of the layers may not be observed because they are too weak. In general, the strongest layer line ($Z = Z_1$) corresponds to $q = 1$ and its spacing $P = 1/Z_1$ would then be the pitch of the helix (as already stated). The spacing of the meridional reflection $h = (1/Z_0)$ gives the unit height. The ratio of the two gives at once the unit twist $t\,(= h/P)$ or the number of units per turn $n\,(= P/h)$. Although these are inherent in the earlier formulæ, they are again explicitly given here to emphasize the simple relationship between the diffraction pattern and

the elements of the helix. In fact, if the two halves of Eqn. (3) are combined, we get

$$Z = mZ_0 + q\,(tZ_0) = m \cdot \frac{1}{h} + q \cdot \frac{1}{P} \tag{20}$$

where m and q are integrals. Thus, the prominent layer lines in the diffraction pattern occur at positions related to the reciprocal of the unit height and of the pitch. The so-called repeat spacing along the helical axis is nowhere in the picture.

The extension to the case of the coiled-coil is obvious and combining the two halves of Eqn. (15), we get

$$Z = mZ_0 + p\,(t_0Z_0) + q\,(t_1Z_0) = m \cdot \frac{1}{h} + p \cdot \frac{1}{P_0} + q \cdot \frac{1}{P_1} \tag{21}$$

where P_0, P_1 are now the pitches of the major and the minor helices. The positions of the layer lines now depend only on the reciprocal of the unit height and the reciprocal of the pitches of the major and minor helices.

The great advantage of the new formalism is that one is able to get out of the restrictions imposed by rational relations. It is obvious that the degree of coiling or supercoiling, when it exists, is determined only by interatomic valence forces and that, so long as no rigid ordering imposed by a crystal lattice exists, it is not limited by any integral or rational conditions. This view-point has greatly helped in solving the helical structure of feather keratin (to be published elsewhere). So also even in the collagen structure which has been considered in the past by the author himself to have exactly 10 residues in 3 turns, a more careful study seems to indicate that no such *exact* relationship exists, although actually the number of residues per turn observed is close to it (also to be published elsewhere).

This turning away from the law of rational numbers was started by Pauling and co-workers (1951) when they put forward the idea of non-integral helices, *i.e.*, that helices with n not equal to an integer can occur in protein structures. It now appears that there is nothing sacrosanct in the use of rational numbers and that any helix can occur in a fibre structure and that it need not have even a rational number of residues per turn.

It must be stated however that if such helices form a crystal lattice with rigid interhelical bonds which are strictly repeated by translational symmetry, then the rules of classical crystallography do hold and no values other than $n = 1, 2, 3, 4$ or 6 can occur in such a structure.

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