Structural Information of Long-Chain Fatty Material Obtained Rapidly and Conveniently by the Use of X-Ray Diffraction Studies of Single Crystals of Urea and Thiourea Adducts

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Abstract

X-ray diffraction patterns of single crystals of urea or thiourea adducts of long chain fatty material (channel type of inclusion compounds) yield diffuse, continuous layer lines instead of the usual spots obtained with ordinary crystals. These lines result because adduct crystals have order in one dimension only, (the channel direction) whereas in ordinary crystals there is order in three dimensions which yields spots.

These continuous layer lines offer possibilities for structure determination of the included molecule (guest). From their spacing, i.e., their distance from the equator, the length of the guest can be quickly calculated. This in turn can give information on 1) degree of branching, 2) *cis-trans* isomerism if the substance is unsaturated, and 3) molecular weight of the guest. From an evaluation of the intensities of the various orders of the layer lines, the position of a substituent group (such as a methyl, hydroxyl or keto group) on the hydrocarbon chain of the guest can be determined.

Introduction

THE USEFULNESS of the channel type of inclusion compounds known as urea and thiourea adducts can be appreciated from the following tabulation:

1) Adducts can be used to separate complex mixtures by virtue of the molecular shape of the components. Thus urea will separate straight and monomethyl branched chain material from more highly branched material (1). Thiourea, because of its slightly larger channel diameter, can form adducts with somewhat more branched material to enable separation from even still more highly branched material (2). It has been possible to separate natural mixtures into four fractions: a) components adducting with urea only, b) components adducting with urea *and* with thiourea, c) components adducting with thiourea only, and d) components not adducting with either urea or thiourea (3).

2) Adducts can be used for storing labile compounds. Unsaturated fatty acids for example, can be stored as urea adducts for long periods of time without oxidative deterioration (4). Thus essential fatty acids can be fed to animals as urea adducts without harmful effects from urea (5). Insecticides can also be administered in this way (6). Thiourea adducts of many common insecticides are prepared (DDT, chordan, methoxychlor, etc.) and are easily handled as non-corrosive powders. On decomposition of the adduct with atmospheric water, the insecticide is released in effective concentration and the thiourea can be utilized by the plants.

3) Optical isomers can be separated by means of urea adducts. Because they crystallize in either right hand or left hand screw-shaped spirals, urea adducts have been used to separate optical isomers, and these compounds, such as 2-bromooctane, have been particularly difficult to separate by other methods (7). Thiourea adducts cannot be used for this purpose because they crystallize in a system that has no screw axis of symmetry.

4) Stereospecific polymerizations can be made to take place in urea and in thiourea adducts. Because the guest molecules are stacked one after the other in the channels of urea or thiourea adducts, the ends are in a definite stereochemical relation. When these ends are made to react, a stereospecific polymer results (8,9).

5) Another use of urea and thiourea adducts—the subject of this paper—results from the fact that the guest molecules in single crystals diffract X-rays to yield continuous layer lines. The spacing of these lines enables easy computation of the length of the channel occupied by the guest. The relative intensities of the successive diffraction orders of these lines enables the determination of the position of a substituent group on the hydrocarbon chain of the molecule.

Reports that continuous layer lines are produced by X-ray diffraction of single crystals of urea or thiourea adducts have been made simultaneously and independently by four different investigations (10,11,12,13).

Origin of the Continuous Layer Line Produced by X-ray Diffraction of Adducts

These lines originate from the unique manner in which the guest molecules are stacked in the channels of the adducts. To understand this it is necessary to consider first, the structure of urea and thiourea adduct crystals, and second, the fundamental manner in which X-ray diffraction itself occurs.

The structure of urea adducts. According to the X-ray analysis by Smith (11) and by Hermann and Renninger (14) these adducts consist of a network of hexagonal channels, the cross section of which can be likened to a honeycomb. The channels are open at both ends, and the guest molecules lie within them, held in place by van der Waals forces of attraction. Generally, the longer the hydrocarbon chain of the guest molecule, the more stable is the adduct. The packing of the molecules in one channel usually does not match up with the packing of the molecules in adjacent channels in a definite way, so that effectively each channel behaves as a one dimensional crystal. The walls of the channels are formed by the urea molecules. The oxygen atoms occupy the corners of the hexagon and the $C(NH_2)_2$ groups, which are hydrogen bonded to the O-atoms, constitute the faces. The orientation of the plane of the urea molecule is such that a line drawn through the two N-atoms is parallel to the long channel axis. Six urea molecules make up a unit cell, and the length of such a cell in the channel direction (c-periodicity or c-axis), as

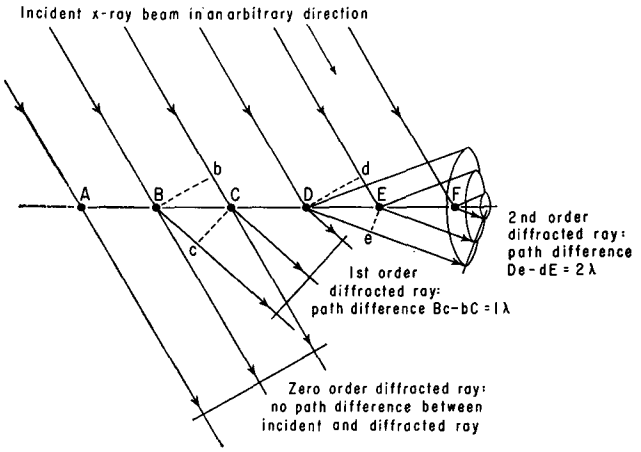


FIG. 1. Conditions for diffraction of a monochromatic X-ray beam impinging on a row of atoms, A,B,C, etc., spaced at regular intervals. Note that diffraction occurs only when the path difference of the incident and diffracted ray is an integral number of wavelengths, λ.

determined by Smith, is 11.005 ± 0.005 Å. The hexagonal cross sectional distance of the unit cell from one face to the other was determined by him as 8.230 ± 0.004 Å. The thickness of the urea molecules making up the channel walls leaves somewhat less space available for the adducting molecules (~ 5 Å). This is almost ideally suited for packing in a straight hydrocarbon chain. Each of the six molecules of urea (whose planes make up the hexagonal faces of a unit cell) is displaced one-sixth the length of a unit cell in the channel direction as one goes around the cell from one face to the next consecutive one, thus making up, in effect, a hexagonally shaped spiralling wall with spiralling spaces in between (see Fig. 8 of Ref. 15). In addition, since the van der Waals approach of molecules can be somewhat variable, the space available to the guest molecule is made adaptable to the guest's packing requirements. Thus there could be space enough to pack snugly into the channel a methyl group substituted on a hydrocarbon chain or a carboxyl group or several *cis* double bonds.

The structure of thiourea adducts. According to Hermann and Lenné (10) and to Lenné (15) these adducts also consist of a network of hexagonal channels, open at both ends, with the guest molecules lying in the channels also packed as one-dimensional crystals. Here the thiourea molecules form the walls with the S-atoms occupying the corners and the $C(NH_2)_2$ groups, hydrogen bonded to the S-atoms, the faces. The orientation of the plane of the thiourea molecule is such that a line drawn through the two N-atoms is parallel to the long channel axis.

The channels differ from those of the urea adducts in the important respect that besides being somewhat larger, they have alternating maximum and minimum zones (two of each per unit cell), giving rise to a series of bulges and necks, as in a bulb condenser. This is in contrast to the uniformly spiralling channel walls as in the case of urea adducts, and results from the fact that at one-half a c-period, 3 S-atoms lie in a plane perpendicular to the c-axis. Lenné states that the maximum channel diameter available for adduct formation is approximately 7.4 Å (at the plane of the 3 S-atoms) and the minimum approximately 6.4 Å. The repeat distance along the channel, i.e., the c-axis of the unit cell, is 12.5 Å.

In addition to these fundamental structure types for urea and for thiourea adducts a case has been found where a molecule has crystallized with urea according to the crystal system of the usual thiourea adduct (24). The molecule in question was isoamyl heptanoate. In the meantime other cases of this kind have been found by Lenné, (personal communication).

X-ray diffraction of adducts, (16). If a parallel beam of monochromatic X-rays from some arbitrary direction is allowed to impinge on a row of atoms spaced at regular intervals, radiation will be scattered in all directions by each atom because the energy absorbed will force the electrons of the atom to oscillate. The resultant radiation from the whole row of atoms will not be equally intense in all directions, for in most directions the rays from each atom will be out of phase and will cancel out. Only in a few directions will the rays be completely in phase and reinforce each other. This condition results when the difference in path length between the rays emanating from any two atoms is equal to an integral number of wavelengths of the radiation used (Fig. 1). If this difference is one wavelength, we have first order diffraction; if it is two wavelengths, we have second order diffraction, etc. The undeflected ray can be considered as zero order diffraction. The

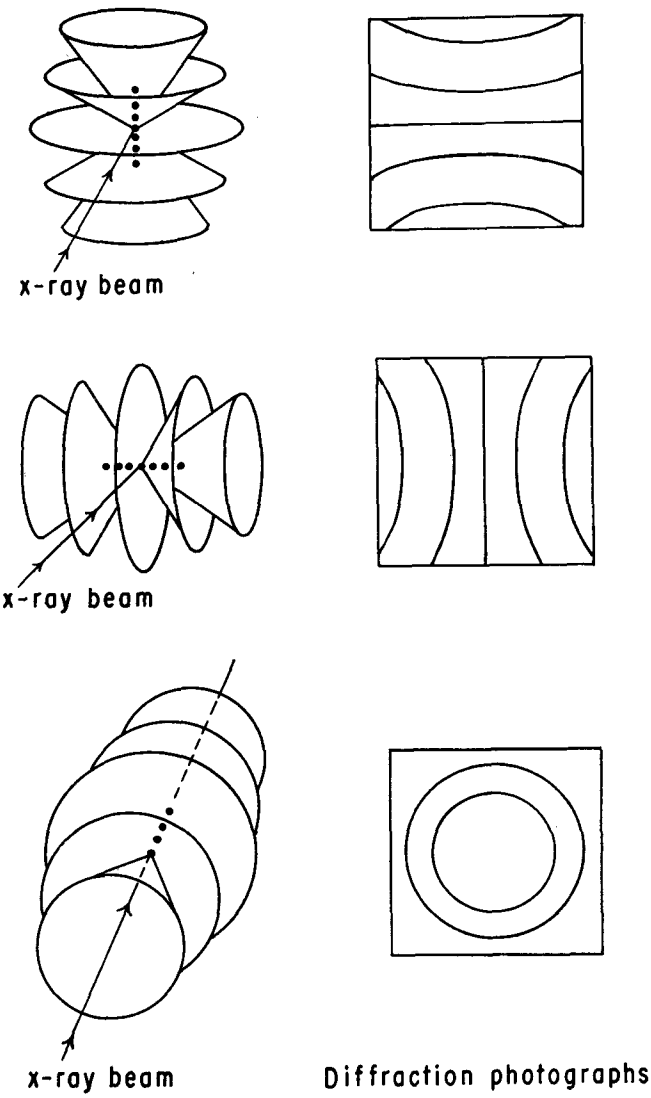


FIG. 2. Cones of diffraction of monochromatic X-rays from a one-dimensional crystal oriented in three different directions. Note diffraction photographs show continuous layer lines in every case. This is the type of diffraction pattern one gets from a urea or thiourea adduct.

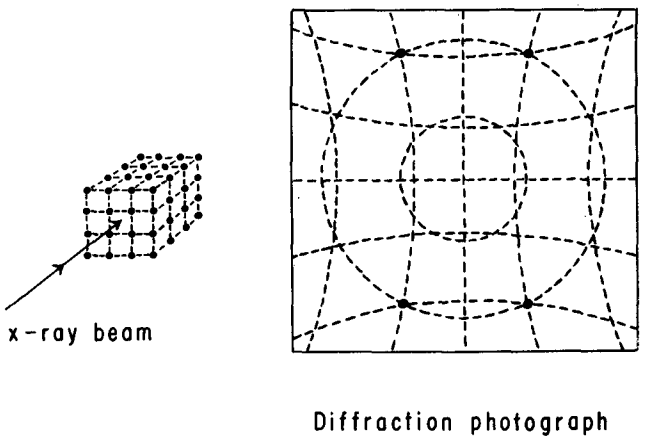


FIG. 3. Diffraction from a two-dimensional plane of atoms by monochromatic X-rays. The cones of diffraction in each direction are indicated by dotted lines on the diffraction photograph. Only the intersections of the cones are visible as is emphasized by the heavy dots on the diffraction photograph.

diffracted ray is not limited to the plane of the paper but forms a cone. It should also be noted that for a given direction, reinforcement may still occur if X-rays of continuously varying wavelengths are used. Such radiation (called "while" radiation) is present when X-rays are generated in the usual manner, i.e., by bombardment of a target element by electrons in a high voltage field.

Cones of radiation for various orders of diffraction from a row of atoms when the incident beam is perpendicular or parallel to the row is shown in Figure 2. When the cones intersect with a plane, as, for example, a photographic plate, they form hyperbolas or circles as indicated. These types of diffraction patterns occur when the diffracting atoms have order in one dimension only. As indicated above, we are calling this type of order a one-dimensional crystal, and this is the type of order we find in adducts.

If there is order in two dimensions only (i.e., so that there is a regular array of atoms in this plane, but these atoms do not line up with the corresponding atoms of other parallel planes to form some kind of a regular three-dimensional pattern), then there would be diffraction of the type indicated in Figure 3. Spots would appear where the cones coming from two directions intersected. Diffraction in all other areas would be cancelled.

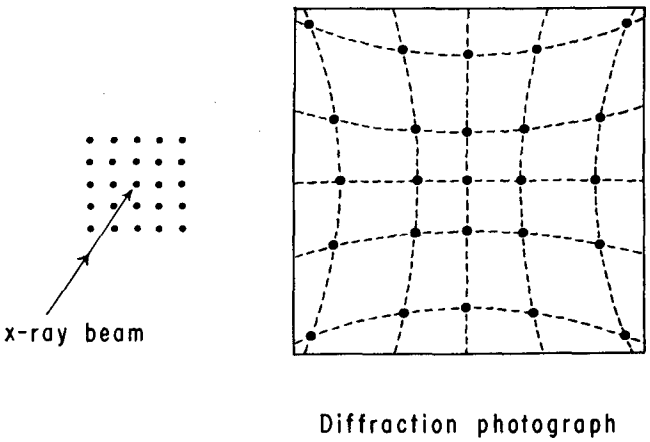


FIG. 4. X-ray diffraction (monochromatic radiation) from a three-dimensional network of atoms as is usually found in crystals. Only when the cones of radiation in three dimensions intersect by chance will a diffraction spot appear. The invisible cones are indicated on the diffraction photograph by dotted lines and diffraction spots by heavy dots.

If there is order in three dimensions, which is the usual case for ordinary crystals, there would be the same requirements for diffraction in two directions *plus* the requirement that the cones of reflection for the third direction would have to coincide with spots already formed from the intersection of cones in two directions. This will happen only by chance (Fig. 4). Usually no diffraction will occur, unless the crystal is moved during the exposure.

Figure 5 is a typical X-ray diffraction pattern of an adduct crystal (urea as host, pelargonic acid as the guest). Copper radiation was used and the crystal was not moved during exposure. The continuous layer lines are due to diffraction by the guest molecules in the adduct channels, the molecules being stacked, as was discussed above, as one-dimensional crystals. Whereas these *lines* are produced (See Figs. 1 and 2) by the characteristic copper radiation ($\lambda CuK_\alpha = 1.54$ Å) the *spots* are "Laue-reflections" of the three-dimensional host structure produced by the white radiation that is always present in an X-ray beam unless it is suppressed by special monochromatization. The relative orientation of X-ray beam, needle axis and photographic plate was as depicted in Figure 2, top. The numbers 1,3,4 and 6 on the print indicate continuous layer lines of the 1st,3rd,4th and 6th order of diffraction.

Figure 6(a) gives further insight into the origin of these lines and shows how they can be used to calculate the molecular length (channel length) of the guest. The points X_1,X_2,X_3, etc., represent the same atom of each of a sequence of guest molecules A,B,C, etc., i.e., the first atom of A, the first atom of B, the first atom of C, etc., or the second atom of each of these molecules, or the j^{th} atoms. In the figure, we chose a periodic sequence of terminal atoms of the guest molecules, but the same periodicity would result had we chosen a sequence of all the second atoms from the end, or all the j^{th} atoms from the end. Each of these periodic sequences of atoms produces cones of diffraction, all of which register on the film. Thus, these lines represent the periodicity or length of channel occupied by the molecule.

We can compute this length of channel, L_c, occupied by the guest, by noting that diffraction will occur only for successive points of the periodic sequence where the difference in path length is equal to $n\lambda$, where n is an integer, being 1 for the first order of diffraction, 2 for the second, etc.

$$L_c = \frac{n\lambda}{\sin 2\theta} \qquad [1]$$

where 2θ is the angle the j^{th} order diffracted ray makes with the zero order diffracted ray. In accordance with common usage, we are calling the "glancing angle," θ, and the diffraction angle 2θ. Figure 6(b) shows how 2θ can be obtained from experimentally measurable distances, namely, the crystal to film distance, f, and the line distance, s.
Thus,

$$\tan 2\theta = \frac{s}{f} \qquad [2]$$

and

$$L_c = \frac{n\lambda}{\sin(\tan^{-1}\frac{s}{f})} \qquad [3]$$

Since n can be recognized from the film (see, for example Figure 13, the diffraction photograph for 9-keto-palmitic acid, where the n values are marked)

and λ is known from the source of monochromatic x-radiation used, we can calculate L_c directly. A sample calculation illustrating the use of these formulas is given at the end of the experimental section.

Evaluation of the intensities of the different orders of the continuous layer lines. It was observed empirically that when the position of a substituent such as a methyl, hydroxyl, or keto group was systematically varied on a hydrocarbon chain, a correlation could be made between the intensities of the various orders of the continuous layer lines formed from X-ray diffraction patterns of single crystals of urea adducts, and the position of the substituent (17). It was also observed that the intensity distribution of these lines could be calculated from theoretical considerations, and further that the relative calculated values matched quite well with a visual comparison of the intensities of these lines. Thus it should be possible to compute the intensity distribution of continuous layer lines of any guest and to compare this distribution with that of an unknown compound without having any reference material.

In this section we will consider the theoretical factors that affect the intensity distribution of lines as related to this problem. In a later section we will present an example of how the intensities of lines can be calculated as well as results and applications.

The main factors that affect the intensities of spots or lines due to X-ray diffraction are tabulated. Only items 1,2, and 3 of the continuous factors and item 2 of the discontinuous factors are discussed here.

A. Continuous factors
   1. Atomic scattering power.
   2. Polarization factor.
   3. Lorentz factor.
   4. Temperature factor.
   5. Geometrical factors.
   6. Absorption factor.

B. Discontinuous factors
   1. Multiplicity.
   2. Structure factor.

The scattering power, f_j, of an atom when irradiated by an X-ray beam, in first approximation, is proportional to the number of electrons in the atom. It also depends on a) how far the electrons of the atom are separated, b) the angle the diffracted ray makes with the undiffracted ray, and c) the wavelength of X-ray used, because all these factors affect the path differences emanating from the electrons. For adducts in which the guests are made up only of C, H and O atoms, there will be the same number of electrons surrounding the C-atom as there are for the O-atom, i.e., 10 electrons. Thus in first approximation f_j depends only on $\frac{\sin\theta}{\lambda}$ where θ is half of the diffraction angle, i.e., the "glancing angle."

The polarization factor is one which is due to the ratios of the amplitudes of the electrostatic and electromagnetic vector components of the diffracted X-ray beam, which varies with increasing diffraction angle for the unpolarized X-ray beam. Thus the vector component that remains in the plane of the incident and diffracted beam decreases with increasing diffraction angle (becoming 0 at 90°). The vector component normal to this plane does not change. For the general case the intensity is proportional to $(1 + \cos^2 2\theta)/2$.

The Lorentz factor has to do with the spreading of the spot over the range where diffraction occurs. For perfect crystals diffraction occurs only over a few seconds of arc during rotation. Most crystals, however, are not perfect and reflect over some minutes or even as much as a half degree of arc. The correction for this factor is $1/\sin\theta\cos\theta$. Thus the correction,

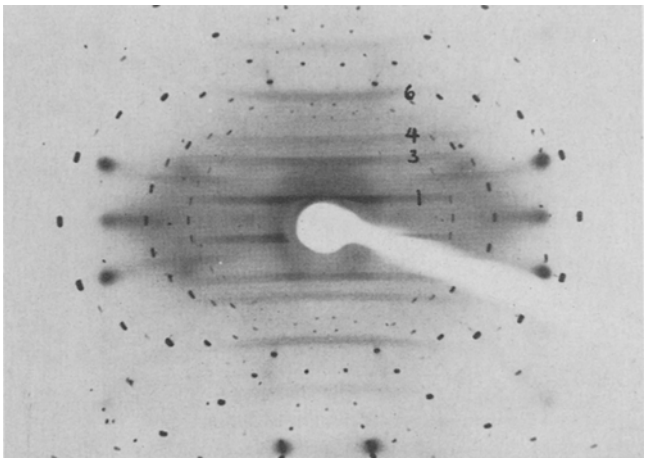


FIG. 5. Laue type X-ray diffraction pattern of a urea adduct crystal of pelargonic acid (crystal not moved during exposure). The photograph was produced with unfiltered copper radiation (40 kv, 15 ma) by exposing for 13 hr. The white image is due to the shield. The lines of order n = 1,3,4 and 6 are marked.

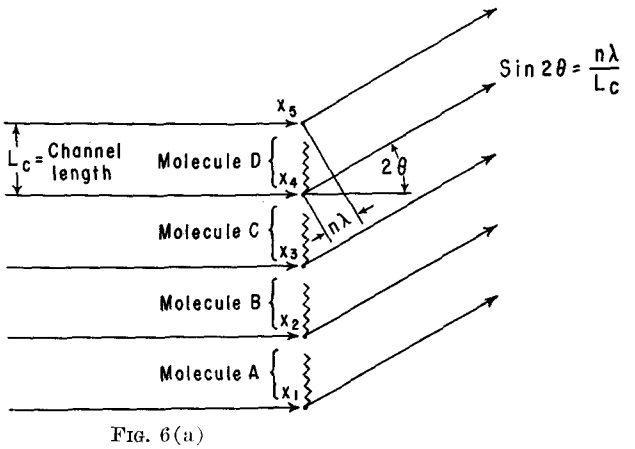


FIG. 6(a)

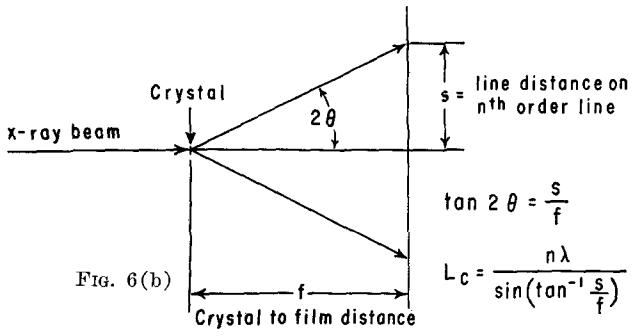


FIG. 6(b)

FIG. 6(a). Conditions for diffraction from all the j^{th} atoms of a series of undecane molecules in an adduct. Long horizontal arrows are incident parallel monochromatic X-ray beams of wavelength λ that strike the j^{th} atom. Oblique arrows are the n^{th} order diffracted rays from this set of atoms. These arrows are a planar section of a cone of radiation that emanates from this sequence of atoms. For this order, cones of radiation emanate from each of the eleven periodically spaced C-atoms in the undecane molecule. Waves from each sequence of C-atoms are added vectorially, i.e., according to phase displacement and amplitude so that the net result is a cone of a characteristic intensity.

FIG. 6(b). Shows how 2θ, hence L_c can be evaluated from experimentally measurable quantities.

C_θ, for the composite polarization and Lorentz factor is

$$C_\theta = \frac{1+\cos^2 2\theta}{2} \times \frac{1}{\sin\theta\cos\theta} = \frac{1+\cos^2 2\theta}{\sin 2\theta} \qquad [4]$$

(Values for C_θ as a function of $\sin\theta$ are tabulated in many books on crystallography, especially Ref. 38.)

The most important factor affecting the intensity distribution of the continuous layer lines is the structure factor, for this factor is sensitive to substituent position on the guest. The intensities of the continuous layer lines at their centers (the Laue indices for this position are $00l$, where "l" would correspond to the "n" in referring to order of diffraction in the general treatment above) are given by

$$I_{c(00l)} = C_\theta \cdot |F_{(00l)}|^2 \qquad [5]$$

where $I_{c(00l)}$ is the relative intensity of the l^{th} order of diffraction, C_θ is the variation of diffraction intensity with diffraction angle as given in formula [4], and $F_{(00l)}$ is the structure factor. The structure factor can be calculated in one of two ways as indicated by formulas [6] and [7].

For the general case

$$F_{(00l)} = \sum_{j=1}^{N} f_j(\cos 2\pi l z_j + i \sin 2\pi l z_j) \qquad [6]$$

where f_j is the atomic scattering factor (assumed here to be uniform for compounds of C, H and O as discussed above), l is the Laue index of reflection order and z_j the coordinate in the chain direction of the j^{th} atom, taking the period length of the adducted compound (repeat distance) as unity. The summation is taken over all N atoms of a repeat unit. Since the intensity of the line is proportional to the square of the absolute value of the structure factor, F, only the sum of the trigonometric terms need be multiplied by its complex conjugate, whereupon the complex terms disappear, i.e., if R represents the sum of all the cosine terms and S is the sum of all the sine terms then

$$F = f_j(R + iS)$$

and

$$(F)^2 = f_j^2(R + iS)\ (R - iS) = f_j^2(R^2 + S^2) \qquad [7]$$

For the case where there are centers of symmetry in the arrangement of atoms of guest molecules, the complex term of the structure factor drops out, simplifying to

$$F_{(00l)} = f_{0.0} + f_{0.5} + 2\sum_{j=1}^{N/2} f_j \cos 2\pi l z_j \qquad [8]$$

if the origin is put at the center of symmetry. The summation is now taken over all N/2 centrosymmetrical pairs of atoms, and the terms $f_{0.0}$ and $f'_{0.5}$ take care of the contribution of atoms to the intensity in case the symmetry centers were occupied by atoms of scattering power f and f′ at the origin, and at the midpoint of the repeat unit respectively. As already mentioned, for compounds of C,H and O, it is assumed that $f = f' = f_j$.

Experimental

If appreciable amounts of material are available, crystals of urea or thiourea adducts suitable for X-ray analysis can be prepared by heating to boiling in a loosely stoppered small vial, 50–100 mg of substance to be adducted with 4–6 ml of a methanol solution saturated with either urea or thiourea (analytical reagents). Up to 1 ml of benzene can then be added to dissolve substances not too soluble in these solutions. The vial is tightly stoppered, wrapped in cotton, and put into a Dewar flask. The Dewar flask, with the wrapped vial in it, is put into a refrigerator and allowed to cool slowly to about 4C over a period of about 16 hr. The supernatant liquid is poured off and the crystals blotted on filter paper. A crystal about 0.2 mm in diameter and about 2 mm long is selected.

Where only smaller amounts of material are available, the procedure is correspondingly scaled down. We have prepared adducts from substances obtained from preparative gas chromatography where the amounts collected were of the order of 0.4 mg. In this case, the material to be adducted is added to a small vial in an appropriate solvent and the solvent blown off with nitrogen. The urea-methanol solution is then added. For 0.4 mg of methyl palmitate, for example, 0.15 ml of a methanol solution saturated with urea was successfully used. The mixture is then warmed, the vial being stoppered loosely at first, then tightly when maximum temperature is reached, then allowed to cool slowly by placing the hot vial in a 400 ml beaker of hot water and allowing the water and vial to cool to room temperature. After a suitable crystal has been found, the remainder of the material can be recovered by decomposing the adduct with water and extracting with hexane or other solvent. Thus, extremely small amounts of material are required.

Differentiation between adduct crystals and crystals of either pure urea or thiourea can be made on the basis of crystal form and index of refraction (18). Urea products are transparent to translucent hexagonal needles (sometimes hollow). Pure urea crystallizes in very clear, transparent tetragonal needles, usually with a wedge-shaped end. To determine whether a given crystal is adduct or urea it usually is sufficient to get an end view of the crystal and to observe the hexagonal form. Thiourea adducts, on the other hand, frequently cannot be differentiated from pure thiourea visually by means of the hexagonal shape, since thiourea itself occasionally crystallizes in pseudohexagonal needles. By measuring the interfacial angles of the crystal on an optical goniometer, it is possible to make such a differentiation quickly. For adducts, the interfacial angles of the hexagonal needles are exactly 60°, while those of pure thiourea are either 54°15′ or 71°30′.

As an additional quick test to determine whether adduct has formed, a comparison can be made of the index of refraction of the two different axes of the crystal with that of a reference liquid (in which the crystal is placed) by observing the Becke line effect through the polarizing microscope (19). To distinguish between urea adducts and urea, a suitable reference liquid is xylene (any isomer). For urea adducts the indices of refraction along both axes are greater than that of xylene. Pure urea shows an index along only one axis greater than that of xylene. For thiourea adducts, a reference liquid consisting of a mixture of iodobenzene and bromobenzene adjusted to give an index of refraction of 1.60 is suitable. Here, in contrast, the indices of refraction along both axes of pure thiourea are greater than that of the reference liquid. Thiourea adducts, on the other hand, have only one axis greater than that of the reference liquid.

If either of these techniques fail, X-ray photographs will quickly differentiate between adduct and

thiourea. Differentiation between urea and urea adducts is rarely a problem as the crystal shapes are decisive.

The continuous layer lines can be revealed by several types of X-ray photographs. Any camera that will take a Laue type X-ray photograph, or a rotation-oscillation photograph is suitable. Powder X-ray photographs do not show the continuous layer line.

We have found it convenient to use the Buerger precession camera. After the crystal is properly orientated, two types of photographs can be taken with one setting of the crystal: 1) a precession photograph, which gives information about the unit cell of the host, and 2) a Laue type photograph which reveals the continuous layer lines.

Our procedure is as follows: After a suitable crystal is selected, it is attached to a very thin glass rod ($\sim$ 2 cm long and 0.2 mm diam) with apiezon, then mounted to the top of a 2-circle goniometer head. The crystal is mounted so that the X-ray beam is perpendicular to the c-axis (needle axis). X-ray source is from copper radiation at 40 kv and 15 ma. Nickel filters are used as required. The crystal is centered with the help of orientation precession pictures of short exposure. To observe the lattice constants of the urea or thiourea framework, zero level precession pictures are taken with the a-axis as precession axis and a precession angle of 30°. To observe the continuous layer lines the Laue technique is used, i.e., crystal held in a fixed position relative to the X-ray beam. The exposure time varies from 12–48 hr depending upon such factors as the quality and size of the crystal and the diameter of the collimator used.

To determine the film to crystal distance most accurately, the camera is calibrated with crystals of known crystallographic properties, e.g., quartz.

To measure the line distances, the films are fastened with Scotch tape to a light box equipped with a viewing arm. The latter can be slid along a side metal ruler so that, by means of a vernier, distances to 0.01 mm can be measured. Since measurements to 0.1 mm are frequently sufficient, these can be done with an ordinary ruler. Correction for film shrinkage or expansion is made by exposing on to the film, light from two pinholes, where the distance between the pinholes is accurately known. By comparing the measured film distances between the pinhole spots with the accurately known distance across these holes, one can compute the amount of shrinkage or expansion.

To give an idea of the accuracy of line-distance measurements and the L_c-values derived from them, the evaluation of a photograph of pelargonic acid (similar to that of Fig. 5) follows.

Three lines were chosen for measurement: 006,004, and 003 (these are the 6th,4th, and 3rd orders of diffraction respectively). For line 006, eight replicate readings were taken of the length 006–00$\bar{6}$, i.e., the height of the line above and below the equator or zero order line. These readings were (in mm) 43.08, 43.21,43.43,43.19,43.18,43.32,43.28, and 43.31, giving an average of 43.25. In a similar manner the average of five replicate readings for 004 and 003 were 27.58 and 20.34.

Correction of these readings for film shrinkage were made as follows: The distance from one light spot to the other was read as 120.89,120.91, and 120.91 mm in three replicate readings or an average of 120.90. The actual distance between the holes (producing these spots) in the metal film holder was accurately measured as 121.50 mm. This means that in 121.50 mm of film the shrinkage was 121.50−120.90 = 0.60 mm. This means that for 43.25 mm of film the true reading, had no shrinkage occurred, would be $43.25 + 43.25 \times 60/121.5 = 43.46$. The other two readings when corrected become 27.72 and 20.44 mm respectively.

The film to crystal distance for this camera was calibrated to exactly 60.00 mm. Therefore, for the 006 line $\tan 2\theta = \frac{43.46/2}{60.00} = 0.3622$; $2\theta = 19.90°$ and and $\sin 2\theta = 0.2729$. Since the wavelength λ used in this work was 1.5418 Å, L_c for 006 would be $\frac{6 \times 1.5418}{0.2729}$ 27.37 Å. For 004 and 003, L_c is 27.40 and 27.52 Å respectively, all of which check rather nicely. These values of L_c are the dimer length of pelargonic acid.

Structural Information Gained by the Measurement of the Channel Length of Guest Molecules

Channel Length. As described earlier, measurement of the distance between continuous layer lines formed from Laue type X-ray diffraction photographs of single crystals of urea or thiourea adducts provide a way of determining the length of channel occupied by the guest molecule. This chanel length is related to the molecular length of the guest, but these terms need clarification. Furthermore, a number of factors affect these lengths.

Figure 7 illustrates schematically the packing of uncoiled hexane molecules in urea and in thiourea adducts (20). The largest distance from the nuclei of the "terminating atom" (21), we shall call the skeletal length, L_s. The skeletal length plus the van der Waals radii of the terminating atoms will then be the van der Waals length, L_v. As Figure 7 shows, L_v does not have to be identical to the c-period of the guest, namely, the length actually measured by experiment, which we are calling L_c. Normally L_c will be smaller than L_v because of tilting and overlapping. Thus

$$L_c = \text{proj } L_s + \text{proj } A_v \qquad [9]$$

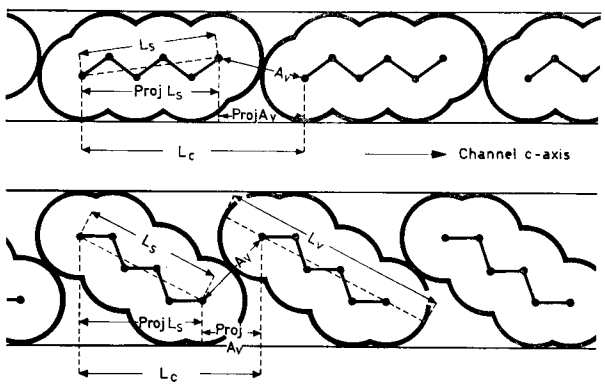


FIG. 7. Upper: A schematic representation of uncoiled hexane molecules stacked in channels of urea adduct. Lower: Same for thiourea adduct. For sake of clarity, the adducted molecules are drawn here as all in one plane but they need not be so in the adduct.

L_v = van der Waals length (i.e., fully extended length); L_s = skeletal length; A_v = van der Waals approach, i.e., closest approach of terminal atoms; L_c = channel length of the molecules; proj = projection of various lengths on the channel axis. See formulas 9, 9a and 10 in text. Reproduced by permission of Z. Kristallographie.

where "proj" means the corresponding projection on the c-axis, and A_v is the distance between the ends of the skeletons at their nearest approach, i.e., the van der Waals approach. Since we are generally dealing with long molecules the difference between L_s and proj L_s is negligible. Thus,

$$I_c = L_s + \text{proj } A_v \quad [9a]$$

Overlapping results because molecules usually pack in a manner to conserve space. Consequently, the direction of A_v is usually not parallel to the channel axis. The difference between A_v and its projection on the c-axis results in a shortening of L_c and is due to the overlapping of ends. We designate it as L_o. Thus,

$$L_o = A_v - \text{proj } A_v \quad [10]$$

These length relationships as well as others involving coiling (to be discussed next) are diagrammatically shown in Figure 8.

In Figure 7, the hexane molecules were sketched in their most extended form. In the adduct, however, a molecule may coil if there is space to do so. We shall designate the resulting shortening due to coiling with the symbol S which in urea or thiourea would be designated S(U) or S(TU) respectively.

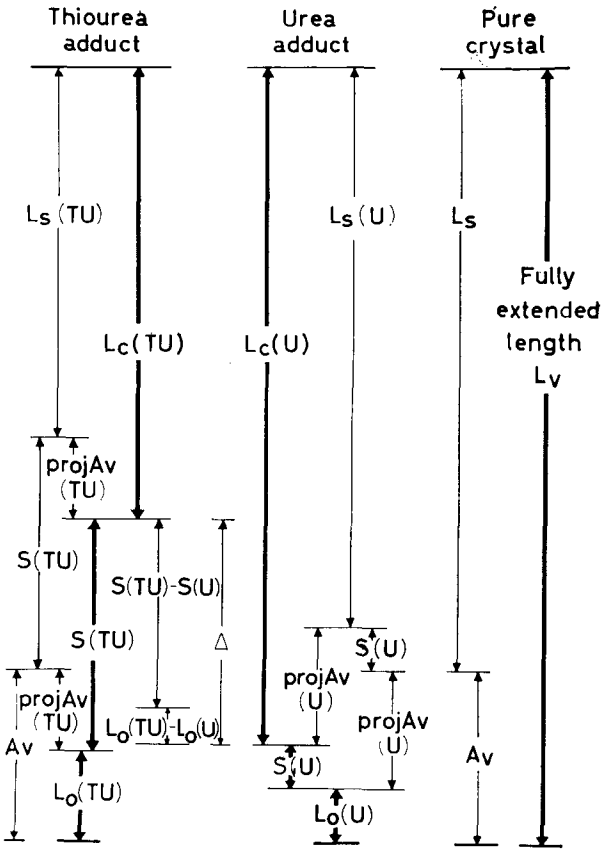


FIG. 8. Diagramatic representation of length interrelationships in the three systems indicated (see also Fig. 7). The symbols (U) and (TU) represent a length in the urea or thiourea adduct respectively. Note that $L_c(U) = L_v - S(U) - L_o(U)$ and $L_c(TU) = L_v - S(TU) - L_o(TU)$, (heavy lines). Note also that $L_s(U)$ or $L_s(TU)$ are shorter than L_s by an amount S(U) or S(TU). This represents the respective amounts of coiling in the two systems. Note also that L_c in either system (heavy lines) $= L_s + \text{proj } A_v$. Note that Δ, the difference in the measured channel lengths of a molecule in the two systems, represents shortening due to additional coiling and additional overlap in going from the urea to the thiourea system. Finally, note that $L_o(U) = A_v - \text{proj } A_v(U)$ and $L_o(TU) = A_v - \text{proj } A_v(TU)$. Reproduced by permission of Z. Kristallographie.

A bond will be called coilable if its projection on the channel axis can become smaller by rotations about other bonds of the same molecule. As indicated in Figure 9 we define the number of coilable bonds of adducted molecules as $N = n - 2 - b$, where n = the number of C-atoms in the longest carbon chain and b = the number of branched groups in that chain. Thus, decane will have 2 coilable bonds and a methyl branched decane will have 7.

The above factors do not consider any "stretching" or "compressing" of a molecule if it is locked-into the regularly spaced structural features of the channel walls. The occurrence of such a phenomenon may be indicated when the c-periodicities of host and guest are in a rational relation, i.e., their ratios are expressible in small whole integers. That such a phenomenon occurs especially in thiourea adducts was shown by Lenné (15). When the guest molecule is not locked into the host framework in this manner it will be here described as irrationally adducted.

Channel Lengths of Molecules in the Urea System. In data to be published, we shall present L_c measurements of urea adducts of the normal paraffins given in Figure 10. Within the limit of error of our measurements, these values all fall on a straight line which can be expressed by the formula

$$L_c = 1.260n + 2.48 \quad [11]$$

where n equals the number of C-atoms in the chain. All the n-paraffins investigated by us (i.e., to n-hexatriacontane) adducted irrationally, thus their packing is independent of the host periodicity.

We can express equation [11] in terms of equation [9a] as follows:

$$L_c = \underbrace{1.260(n-1)}_{I_s} + \underbrace{1.260 + 2.48}_{\text{proj } A_v}$$

Therefore, proj $A_v = 3.74$ Å. Using 4.06 Å as the van der Waals approach, A_v, for two methyl groups (22) we can compute an overlap for two methyl end

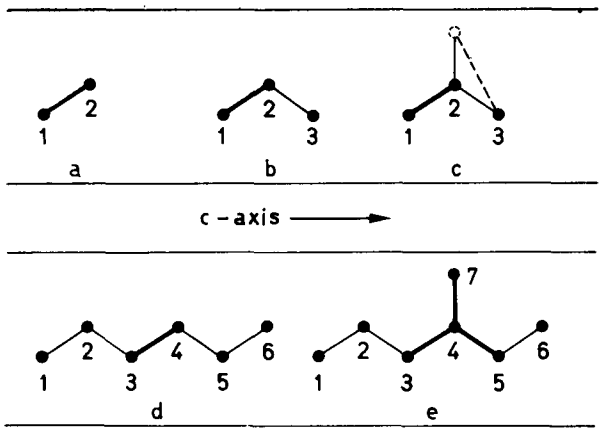


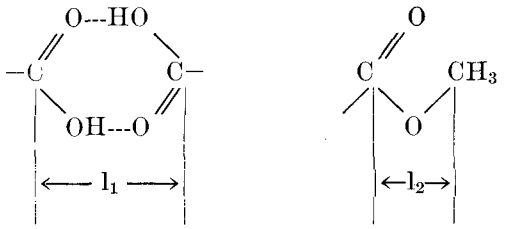
FIG. 9. Diagram to illustrate what we are calling coilable bonds (thin lines), the number of which is considered to be responsible for decrease in channel length of a molecule from that of the fully extended length, L_s. Note that rotation about C_1–C_2 in *a* produced no decrease in its projection on the c-axis, whereas, rotation of C_1–C_2 in *b* does, as depicted in *c*. Thus two C-atoms give no coilable bonds and three C-atoms give one. Generalizing, we define the number of coilable bonds, N, in unbranched chains to be $n-2$ where n is the number of C-atoms, i.e., *d* would have four coilable bonds since we arbitrarily hold one bond fixed (heavy line); any bond could have been chosen as the fixed one. Introducing a branched methyl group as in *e* causes the structure C_3,C_4,C_5,C_7 to take up a rather fixed position in the channel reducing N by one more, i.e., N now is three. Reproduced by permission of Z. Kristallographie.

groups in the urea system with the use of equation [10]

$$L_o = A_v - \text{proj } A_v = 4.06 - 3.74 = 0.32 \text{ Å}.$$

By means of formula [11] the alternate C to C distance of an adducted normal hydrocarbon chain is 2.520 Å. Using 1.526 Å for the C–C bond length and 113° for the C–C–C bond angle (23), the alternate C to C distance is 2.550 Å in a *non*-adducted normal hydrocarbon chain. Thus the difference in the projections onto the long axes of molecules which are not adducted with urea but are fully extended and those that are adducted in urea is 1.275 − 1.260 = 0.015 Å per C–C bond. This small difference we attribute to coiling (20).

Since the deviations from a fully extended molecule as manifested in coiling and in overlapping of ends in the urea system can be dealt with on a quantitative basis, the channel lengths of parts of molecules such as the carboxylic acid dimer group, l_1, (fatty acids adduct as dimers), or the methyl ester group, l_2, can be calculated:



From the channel lengths of a number of homologous fatty acids (20,24) the ones investigated were found to adduct irrationally. The data can be expressed by formula [12], (Fig. 11)

$$L_c = 2(1.260n + 2.39) \qquad [12]$$

where L_c is the channel length of the dimer and n is the number of C-atoms in the monomer. By an extrapolation analogous to the one used above to establish proj A_v for hydrocarbons, when $n = 1$ in formula [12], $L_c = 7.30$ Å. This represents the sum of proj $A_v + l_1$, and since proj $A_v = 3.74$ Å, $l_1 = 3.56$ Å. The best data we are aware of for comparison with non-adducted carboxyl groups are those determined on vitamin A acid. Here $l_1 = 3.89$ Å (25). Considering the extra thickness the carboxyl group would have when the van der Waals radii of the constituent atoms would be added to it, and considering also the small channel diameter of the urea channel (~ 5 Å), the dimer carboxyl group is most likely situated so that the subtended distance l_1 is in the channel direction. Our comparatively low value of 3.56 Å would indicate one of two possibilities or both to be operative: a) the two carboxyl groups take up a staggered or twisted position diminishing the channel length of the dimer group, and b) The snugly fitting carboxyl dimer group may impose an additional amount of coiling to shorten the hydrocarbon chain.

To illustrate another type of calculation the channel length of the methyl ester group, l_2, can be determined by comparing the L_c values for methyl octadecanoate, 27.42 Å, with n-eicosane, 27.68 Å. The difference, 0.26 Å, represents the difference between the $-\overset{\overset{O}{\|}}{C}-O-CH_3$ group and the $-CH_2-CH_2-CH_3$ group. Since the channel length of the latter is 2.52 Å, (formula 11), $l_2 = 2.52 - 0.26 = 2.26$ Å. This value can also be computed from data (24) obtained from channel length measurements of a number of homologous n-methyl esters presented in Figure 11, whence

$$L_c = 1.260n + 4.70 \qquad [13]$$

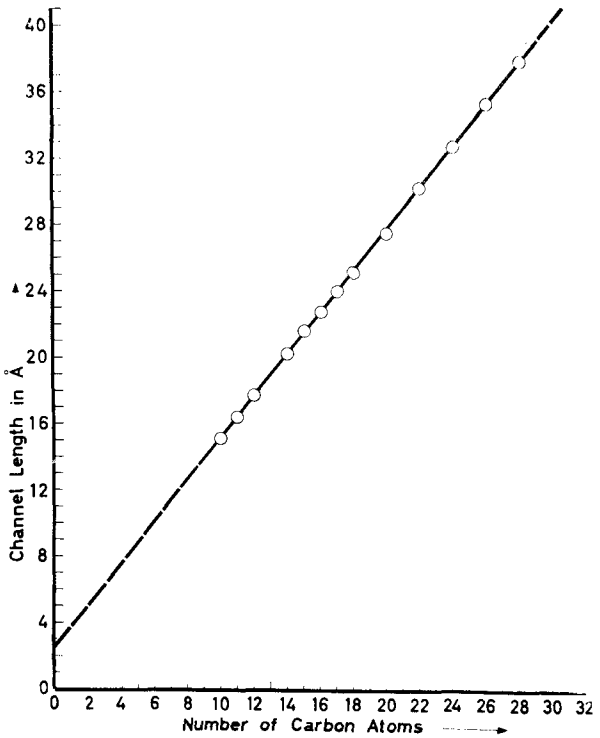


FIG. 10. Channel lengths of a series of straight chain hydrocarbons. These data can be expressed by $L_c = 1.260n + 2.48$ Å, where n is the number of C-atoms in the chain. Reproduced by permission of Z. Kristallographie.

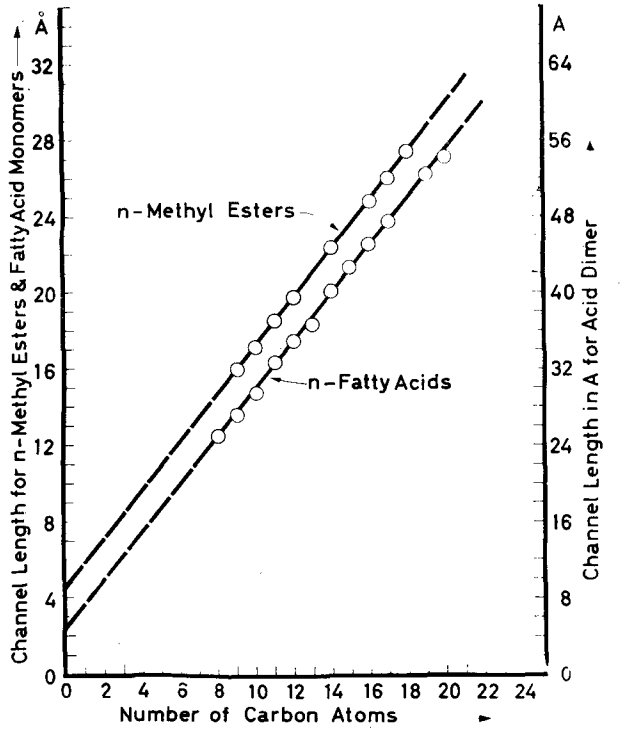


FIG. 11. Channel lengths of a series of n-acids and n-methyl esters. The acids adduct as dimers and can be expressed by the formula $L_c = 2(1.260n + 2.39)$ where n is the number of C atoms in the monomer.

The methyl esters adduct as monomers and can be represented by $L_c = 1.260n + 4.70$ where n is the number of C-atoms in the fatty acid moiety of the methyl ester. Reproduced by permission of Z. Kristallographie.

where n is the number of C-atoms in the fatty acid portion of the methyl ester. The value of L_c for $n = 1$, namely, 5.96 Å = l_2 + proj A_v. For the hydrocarbons, proj A_v was found to be 3.74, therefore, $l_2 = 5.96 - 3.74 = 2.22$ Å. This is somewhat lower than the reported value of 2.47 Å for l_2 in methyl stearate and stems from the fact that the van der Waals approach of 2 ester groups is smaller than that for 2 terminal methyl groups in hydrocarbons (20).

Thus the channel length of a wide variety of molecules can be calculated when the component parts are known. Some of these are listed in Tables I and II. In the urea system, for example, from channel length and molecular weight, it can be determined whether a molecule is straight chained or has a methyl branch.

Channel Lengths of Molecules in the Thiourea System. By methods similar to those described in the urea system, channel lengths of portions of molecules in this system can be computed. A number of examples are given in Reference 20. Partial lengths in this system are also tabulated in Tables I and II.

Because of the alternating wide and narrow channel diameter in this system, molecules are more apt to be locked into the framework. We have found cases where channel lengths of locked-in compounds indicated that the molecules were "compressed," cases where their rational lengths were fortuitously similar to their irrational lengths, and cases where the locking-in produced stretching, real or apparent. (An apparent stretching might be observed if A_v were increased.) Similar observations have been made by Lenné (15). The maximum amount we have observed the channel length of a locked-in compound to deviate from a calculated irrational length is about 0.5 Å.

The Determination of cis-trans Isomerism by Means of Channel Length Measurements. Straight chain molecules containing one or more *cis* or *trans* double bonds form adducts with urea. Some branched chain molecules with one or more *cis* or *trans* double bonds form adducts with thiourea. Since the steric form of the double bond affects the length of the molecule, channel length measurements can be used to determine the number of *cis* and/or *trans* double bonds a molecule contains (18). If the channel length of a molecule having either a *cis* or *trans* double bond is compared with the corresponding saturated analog the shortening per *cis* or *trans* double bond can be determined (Table III). From these and other data we conclude that a *cis* double bond shortens a molecule 0.9 ± 0.1 Å while a *trans* double bond shortens it 0.1 ± 0.1 Å. The method can be applied to mono- and to some polyunsaturated substances. It can also be applied to molecules with certain types of trisubstituted double bonds, especially of the acyclic isoprenoid type. Infrared spectroscopy cannot discriminate between *cis* and *trans* double bonds in this type of molecule.

Both squalene and squalane (perhydrosqualene) form thiourea adducts. This makes it possible to determine the stereochemistry of natural squalene (28,29).

Channel length of squalane [1]	31.06 ± 0.15 Å
Channel length of squalene	30.60 ± 0.15 Å
difference	0.46 Å

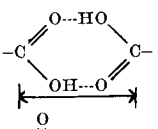
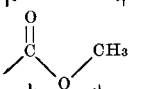
Since 6 *trans* double bonds would shorten squalene 0.6 Å as compared to squalane, natural squalene must be the all *trans* isomer. If 1 *cis* and 5 *trans* double bonds were present then the difference in length would have been 1.5 Å, an amount well out of the limit of error of the method.

That isomers of squalene with *cis* double bonds can be detected in thiourea adducts, can be seen from the length data of various squalene molecules listed in Table IV.

Samples of squalene, Nos. 2 and 3, (30,31) prepared from shark liver oil and purified by distillation, gave the same length as a sample of squalene, No. 4, obtained from human hair fat and purified only by silica gel chromatography (33). The initial and final crystals from the shark liver preparations were found

TABLE I

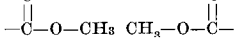
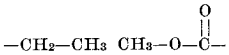
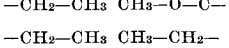
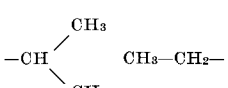
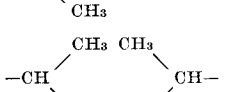
Skeletal Lengths and Shortening Due to Coiling for Parts of Molecules in Different Systems [a]

No.	Portions of molecules considered are subtended by the arrow	Projection on long axis of fully extended molecules (crystal)	Urea adduct		Thiourea adduct	
			L_s(U)	S(U)	L_s(TU)	S(TU)
1		1.275	1.260	0.015	1.100 1.165[b]	0.175 0.110[b]
2		2.55	2.52	0.03	2.20	0.35
3		3.89	3.56	0.33	3.56	0.33
4		2.47	2.22	0.25		
5					3.68	0.11
6					4.85	0.22
7					8.32	0.55

[a] For definition of symbols, see text. For further details as to the origin of these values see Ref. 29.
[b] For coilable bonds in structures 5 and 6 of this Table.

TABLE II

Values of Overlap for Various Combinations of Ends of Molecules in Different Systems [a]

End combinations [b]	Pure substance (crystal) A_v	Urea adduct		Thiourea adduct	
		proj A_v	L_o(TU)	proj A_v	L_o(TU)
	3.30				
	3.68	3.39	0.29	2.39	1.29
	4.06	3.74	0.32	2.86	1.20
		3.74	0.32	2.94	1.12
		3.74	0.32	3.34	0.73

[a] For definition of symbols, see text. For further details as to the origin of these values see Ref. 29.
[b] Random combinations.

[1] Squalane adducted rationally so that it was somewhat "stretched." See Reference 29 as to how an irrational length was accurately determined.

TABLE III *

Channel Length of Some Compounds of Known *cis-trans* Configuration in Urea and Thiourea Adducts

Substance	Carbon chain length	Double bonds	Type of adduct	Channel length in Å	Shortening per double bond	No. of preparations of adducting compounds	Laue films taken	The order of the lines measured	c-axis of host in Å
Stearic acid	C_{18}	none	urea	50.10 [a]	.....	2	6	5th	11.03
Oleic acid	C_{18}	*cis*-9	urea	48.44 [a]	0.83	2	3	5th	10.99
Linoleic acid	C_{18}	*cis*-9, *cis*-12	urea	46.63 [a]	0.87	1	2	5th	10.98
Elaidic acid	C_{18}	*trans*-9	urea	49.82 [a]	0.14	2	7	5th	11.00
Linolelaidic acid	C_{18}	*trans*-9, *trans*-12	urea	49.18 [a]	0.23	1	3	5th	11.00
Methyl stearate	C_{18}	none	urea	27.42	.....	1	6	3rd,4th,5th	.....
Methyl oleate	C_{18}	*cis*-9	urea	26.39	1.03	1	3	3rd,4th,5th	11.01
Methyl eicosanoate	C_{20}	none	urea	29.92 [b]	.....	..	..	.....	.....
Methyl eicosenoate	C_{20}	*cis*-11	urea	28.95	0.97	1	1	3rd,4th,5th	11.00
Methyl decosanoate	C_{22}	none	urea	32.45 [b]	.....	..	..	.....	.....
Methyl docosenoate	C_{22}	*cis*-13	urea	31.47	0.98	1	1	3rd,4th,5th	10.99
Methyl linoleate	C_{18}	*trans*-10, *trans*-12	urea	26.96	0.23	1	2	2nd,3rd,4th,5th	.....
Methyl linoleate [c]	C_{18}	*cis* & *trans* [d]	urea	26.14	0.64 [e]	1	2	2nd,3rd,4th,5th	.....
2,2,4-trimethylpentene	C_5	none	thiourea	8.39	.....	2	3	1st,2nd	12.50
2,4,4-trimethylpentene-2	C_5	*trans*-2	thiourea	8.30	0.09	1	1	1st,2nd	

[a] Dimeric length.
[b] Length obtained by extrapolation from data on methyl esters to be published.
[c] Obtained by alkali isomerization: a mixture approximately 45% *trans*-10, *cis*-12 and 45% *cis*-9, *trans*-11, plus 10% other isomers.
[d] A *cis* and a *trans* double conjugated.
[e] Average shortening.
* Permission to republish this table, taken from Ref. 18, has been granted by the J. Am. Chem. Soc.

to give the same channel length. This shows that the bulk of this preparation was homogeneous. The yield of adduct crystals from squalene from human hair fat was high, and random crystals gave the same length. This also shows that the adduct preparation was quite homogeneous. These facts indicate that not only did the samples from the two different sources give the same steric form, but also that the two purification procedures do not materially alter configuration.

Sample No. 5 was obtained from a chemical synthesis of squalene through the intermediate of a natural nerolidol (32). Sample No. 6 was obtained from the same chemical synthesis of squalene through the intermediate of a synthetic nerolidol (32). Both samples gave channel length indistinguishable from those of natural squalene indicating that the syntheses were successful in yielding the all-*trans* isomer. Since natural nerolidol was used in Sample No. 5, it could have been deduced that natural nerolidol must contain at least some of the *trans* isomer (34).

Samples 7 and 8 were fractions of a chemically synthesized squalene preparation made by Dicker and Whiting (35,36) by coupling two moles of *trans*-geranyl acetone with one of tetramethylene-1,4-bis-triphenylphosphonium bromide. The hydrocarbon isolated from this reaction must have been a mixture of at least three stereoisomers, namely: a) *trans*-10, *trans*-14; b) *cis*-10, *trans*-14; and c) *cis*-10, *cis*-14. The mixture was treated with a saturated solution of thiourea in methanol and an initial crop of crystals was obtained. A portion of these crystals was sent to us for X-ray analysis, (Sample No. 7). Dicker and Whiting treated the remaining hydrocarbon exhaustively with thiourea in methanol until no further adduct formed. The portion of oil not forming adduct was chromatographed on alumina and found by infrared spectroscopy to contain no exo-

$$-CH_2-\overset{CH_2}{\overset{\|}{C}}-CH_2-$$

methylene groups, . This fraction was then submitted to us for analysis. Treatment of this oil with a few drops of benzene and a saturated solution of thiourea in methanol, then cooling the mixture slowly to 4C did, however, yield a further small crop of crystals suitable for X-ray analysis. This is sample No. 8. Since the channel length of Sample No. 7 was the same as that of natural squalene, the material from this synthesis which formed adduct with thiourea most easily must have been the all-*trans*-isomer. Sample No. 8, which did not form adduct so readily, however, gave a channel length definitely indicating the presence of *cis* double bonds (in amount roughly estimated at 20 to 35%).

Three samples of "squalene" regenerated from the hexahydrochloride (30,37) (Nos. 9,10, and 11) also gave length distinctly less than the all-*trans* isomer. No. 11, which was purified via the thiourea adduct (30) and was presumably richer in the all-*trans* isomer, showed a greater length than No. 10.

Squalene hexahydrochloride itself formed a thiourea adduct giving a length of 31.23 Å and a rather low c-period of 12.47 Å. This molecule was definitely locked into the thiourea channels in the manner as described (20) (L_c/c-period = 2.504).

It would be interesting if channel length measurements are able to differentiate between the two steric forms of the cyclopropane fatty acids. Just as in the case of *cis* or *trans* isomers, the channel

TABLE IV

Channel Length of Squalene from Different Sources and Other Data [a]

No.	Substance, Source [b]	Channel length Å	Shortening [c] Å	Host c-axis Å
1	Perhydrosqualene	31.06	0.00	12.52
2	Natural squalene (from shark liver oil)	30.57	0.48	12.53
3	Natural squalene (from shark liver oil)	30.60	0.45	12.56
4	Natural squalene (from human skin)	30.62	0.43	12.52
5	Synthetic squalene (from natural nerolidol)	30.51	0.54	12.54
6	Synthetic squalene (from synthetic nerolidol, i.e., total synthesis)	30.57	0.48	12.54
7	Synthetic squalene (easily adducting with thiourea)	30.60	0.45	12.50
8	Synthetic squalene (difficultly adducting with thiourea)	30.34	0.71	12.49
9	"Squalene" regenerated from the hexahydrochloride	29.24	1.81	12.51
10	"Squalene" regenerated from the hexahydrochloride	29.26	1.79	12.51
11	Sample No. 10, but purified by thiourea adduct formation	30.19	0.87	12.50
12	Squalene hexahydrochloride	31.23	.....	12.43

[a] All values reported are an average of replicate measurements of several films.
[b] One sample of two preparations of perhydrosqualene used in this work and Sample Nos. 2,5,6,10, and 11 were kindly donated by the Research Group of Hoffmann LaRoche & Co., A. G., Basel, Switzerland. Samples No. 7 and 8 were kindly donated by Drs. Whiting and Dicker of Oxford University, Oxford, England. A second sample of perhydrosqualene was prepared by hydrogenation, of purified natural squalene with PtO_2 in alcohol (bp. 162–6° at 0.05 mm), iodine value = 0. No. 3 was prepared as previously described (Ref. 31) as was No. 4 (Ref. 33). No. 9 was kindly donated by Drs. R. G. Langdon and K. Bloch (Ref. 37). No. 12 was prepared according to (Ref. 39).
[c] Shortening = channel length of No. 1 minus that of substance.

TABLE V

Calculated and Observed 00*l* Intensities of Monosubstituted Palmitic Acid Dimers as Urea Adducts

Order of Reflection		1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
Palmitic acid	I_c	151	0	37	1	12	2	2	4	0	5
Palmitic acid	I_o	m	....	m—	....	wk	vwk	vwk	wk	....	wk
4-substituted p.a.	I_c	275	0	15	12	2	2	7	0	2	4
4-keto-p.a.	I_o	st	....	wk+	wk+	wk—	vwk	wk+	vwk	vwk	wk
4-hydroxy-p.a.	I_o	st	....	wk+	m	wk—	wk	m	vwk	wk	wk+
11-substituted p.a.	I_c	94	7	87	2	2	0	3	13	1	3
11-keto-p.a.	I_o	m—	vwk	m+	....	vwk	....	vwk	wk+	....	vwk
11-hydroxy-p.a.	I_o	m+	wk	st	wk	wk	vwk	wk+	m+	wk	wk+
12-substituted p.a.	I_c	80	2	87	8	10	0	0	2	2	13
12-keto-p.a.	I_o	m+	....	st	m—	m	....	....	wk	wk	m
12-hydroxy-p.a.	I_o	wk+	vwk	m	wk	wk	....	....	wk—	wk—	wk

p.a. = palmitic acid; I_o = observed intensity; I_c = calculated intensity; st = strong; m = medium; wk = weak; v = very.
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length of the isomer, where the long chains of the molecule are on opposite sides of the cyclopropane ring, would be expected to be greater than that of the isomer where the long chains are on the same side.

Determination of Molecular Weights by the Use of Channel Length Measurements. If the channel length of the guest and the c-period of the host are measured, then the molar ratio of host to guest can be computed, for the number of molecules of host per unit cell (or c-period) is known. (There are six molecules of urea or thiourea in a unit cell.) If the weight of guest per weight of host is determined

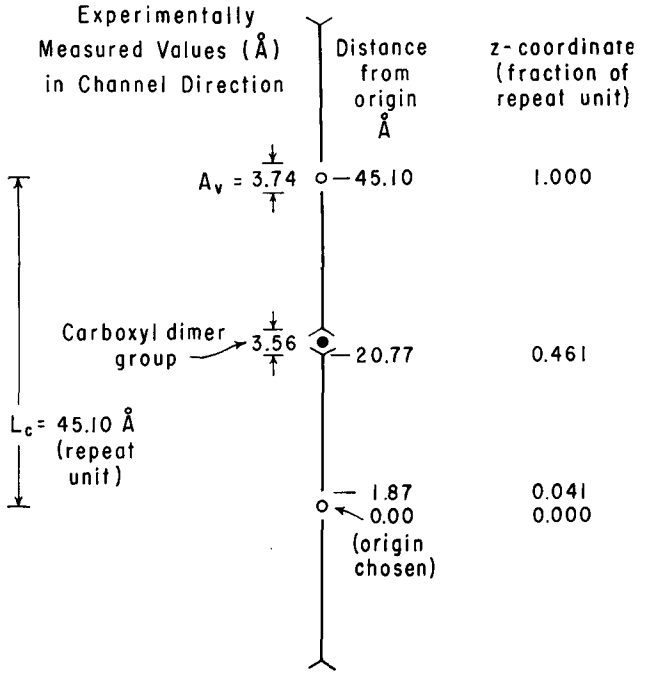


FIG. 12. Schematic drawing of a repeat unit (L_c) of palmitic acid as adducted in urea. One complete dimer is drawn. Note that there are two different centers of symmetry, indicated here as open circles and black dots. The open circles are located midway between the C-atoms of the terminal methyl groups for two adjacent dimers and the black dots are at the centers of the carboxyl dimer group of a single dimer. (This carboxyl dimer group was designated as 1 earlier.) Atoms O_1 are the carbonyl oxygen atoms and atoms O_2 are hydroxyl oxygen atoms of this carboxyl dimer group. Taking a circle as origin the terminal atom C_{16} of a palmitic acid molecule is 1.87 Å from the origin as measured in the channel direction; C_{15} would be 1.87 Å + 1.26 Å = 3.13 Å from the origin and so on, since the C–C distance projected on the channel axis has been measured experimentally to be 1.26 Å as indicated by formula 12. Proceeding to the C_1 atom its distance from the origin is 20.77 Å. For 00*l* intensity calculations the value of the repeat unit has to be taken as "unity," thus the z-coordinate of the C_{16} atom becomes 1.87 Å/45.10 Å = 0.041 and so on. The 3rd column of Table VI contains the z-coordinates of all atoms. (The z-coordinates of the oxygen atoms, O_1 and O_2, have been chosen in accordance with the experimentally determined value l_1 = 3.56, see discussion of formula 12.)

(by decomposing a weighed amount of adduct and isolating and weighing the guest), then the molecular weight of the guest can be found.

Let L_c = the channel length of the guest, and
c-period = the length of a unit cell in the channel direction.

From X-ray data:

$$\frac{\text{moles guest}}{\text{moles host}} = \frac{1}{\frac{L_c}{\text{c-period}} \times 6}$$

From weight data obtained by decomposition of a weighed amount of adduct:

$$\frac{\text{moles guest}}{\text{moles host}} = \frac{\frac{\text{wt guest}}{\text{Molecular wt guest}}}{\frac{\text{wt host}}{\text{Molecular wt host}}}$$

Therefore,

$$\text{molecular wt of guest} = \frac{6 \times L_c \times (\text{Mol wt host}) \times (\text{wt guest})}{(\text{c-period}) \times (\text{wt host})} \quad [14]$$

For example, a sample of the adduct from natural squalene (purified by distillation) was prepared by adding 0.4 g of squalene to 50 ml of a saturated solution of thiourea in methanol, warming the mixture, then allowing it to cool to room temperature and stand for several hours. All the oil droplets of squalene disappeared and crystals of adduct appeared. Excess solution was decanted and the crystals blotted on filter paper. That no thiourea crystals were also present in the adduct crystals was shown by the index of refraction test of random crystal samples (see experimental section). Two samples weighing 232.19 and 189.35 mg respectively were decomposed with 25 ml of water and the oil extracted completely from the mixture with pure petroleum ether. When the solvent was removed with a stream of nitrogen a clear white oil remained which weighed 62.21 and 50.98 mg at constant weight. Pure thiourea recrystallized from methanol and put through the same process gave a negligible blank of 0.01 mg.

Taking the average channel length of squalene as 30.60 and the c-period as 12.54 and the molecular weight of thiourea as 76.12, the values for the molecular weight of squalene are 408.1 and 410.8, respectively. This is in excellent agreement with 410.7 as computed from atomic weights. In this method of determining molecular weight it does not matter whether the guest is locked into the framework of the host as long as both L_c and c-period are measured.

Structural Information Gained by Evaluation of Line Intensities

As described earlier, Laue type X-ray diffraction patterns formed from single crystals of urea or thio-

urea adducts give continuous layer lines which vary in relative intensity with successive orders of diffraction. Furthermore, this variation is sensitive to the position of a substituent on the hydrocarbon chain of the guest. Formulas were also given whereby relative intensities of these lines could be calculated. Table V shows a comparison of the calculated and the observed intensities for several positional isomers of substituted palmitic acids (17).

An example of the calculation of relative intensities of the continuous layer lines will now be given. Figure 12 shows the repeat unit of palmitic acid. As origin, the symmetry center between two dimers was chosen. The z-coordinates of the atoms are derived as explained in the legend of Figure 12.

Since there are symmetry centers formula [8] can be used

$$F_{(00l)} = f_{0.0} + f_{0.5} + 2 \sum_{j=1}^{N/2} f_j \cos 2\pi l z_j$$

There are no atoms at the symmetry centers, i.e., where $z = 0.0$ and $z = 0.5$. The formula therefore simplifies to

$$F_{(00l)} = 2 \sum_{j=1}^{N/2} f_j \cos 2\pi l z_j$$

Since only relative intensities are important here the formula further simplifies to

$$F_{(00l)} = \sum_{j=1}^{N/2} f_j \cos 2\pi l z_j$$

As was discussed above, the f_j values (the scattering power) of the C and of the O atoms can be considered equivalent; therefore, this factor f_j can be taken out of the sum, i.e.,

$$F_{(00l)} = f \cdot \sum_{j=1}^{N/2} f_j \cos 2\pi l z_j$$

For palmitic acid $N = 36$ (i.e., the number of atoms per repeat unit), thus $N/2 = 18$ terms have to be summed up for each order of diffraction, $00l$. This is done in Table VI where l is the 1st, 8th and 10th orders.

Since intensities are proportional to the square of the structure factors:

$$F^2_{(001)} / f^2 = 4.18 \; ; \; F^2_{(001)} = f^2 \cdot 4.18$$

$$F^2_{(008)} / f^2 = 1.13 \; ; \; F^2_{(008)} = f^2 \cdot 1.13$$

$$F^2_{(00.10)} / f^2 = 2.33 \; ; \; F^2_{(00.10)} = f^2 \cdot 2.33$$

From formula [5] the relative intensities $I_{c(00l)}$ can be calculated:

$$I_{c(001)} = C_\theta \cdot F^2_{(001)} = C_\theta \cdot f^2 \cdot 4.18$$
$$I_{c(008)} = C_\theta \cdot F^2_{(008)} = C_\theta \cdot f^2 \cdot 1.13$$
$$I_{c(00.10)} = C_\theta \cdot F^2_{(00.10)} = C_\theta \cdot f^2 \cdot 2.33$$

$C_\theta = \dfrac{1 + \cos^2 2\theta}{\sin 2\theta}$ and f are dependent on θ ("glancing angle"). In addition f depends on the atomic number as already discussed. Both functions are tabulated in the International Tables (38) and are given here for the particular values of θ corresponding to the line distances of 001, 008 and 00.10.

I	II	III	IV	III × IV
$00l$	θ	$\frac{1}{100} \cdot C_\theta \cdot f^2$	$F^2_{(00l)}/f^2$	$I_{c(00l)}$
001	1°	36.2	4.18	151
088	8°3′	3.1	1.13	3.5
00.10	10°9′	2.0	2.33	4.7

To column III: The factor 1/100 has been added to avoid large figures. This is justified since we are interested only in relative intensities.

When a substituent is present on the hydrocarbon chain of palmitic acid, the intensity distribution of the different orders of diffraction change. The case of 9- and 10-keto palmitic acids illustrates this effect well. With these acids the cosine terms for C_9 and C_{10} of Table VI should be taken twice, because O-atoms are also present at these z-coordinates. This causes the intensity distribution for the 1st, 8th and 10th orders to change as is shown in Table VII.

Note the underlined intensity values of Table VII and compare them with the intensity values actually found for these compounds as shown in Figure 13. Especially note that when an O-atom is present on the 9th C-atom of palmitic acid, the relative intensities of the 8th and 10th orders of diffraction reverse themselves if this atom is now placed on the 10th C-atom. In general, some differences could be found for the relative intensity distribution of the various diffraction orders for all the fatty acids when the substituent on the fatty acid chain was systematically varied.

TABLE VI

Calculation of Relative Line Intensities for 001, 008 and 00.10 of Palmitic Acid *

	c-coordinates		8z	10z	cos2 · 1z	cos2 · 8z	cos2 · 10z
	Å	z=Å/41.5					
C_{16}	1.87	0.041	0.332	0.415	0.967	—0.493	—0.861
C_{15}	3.13	0.069	0.555	0.694	0.907	—0.941	—0.345
C_{14}	4.39	0.097	0.779	0.974	0.820	0.181	0.987
C_{13}	5.65	0.125	1.002	1.253	0.707	1.000	—0.019
C_{12}	6.91	0.153	1.226	1.533	0.572	0.150	—0.979
C_{11}	8.17	0.181	1.450	1.812	0.420	—0.951	0.380
C_{10}	9.43	0.209	1.674	2.092	0.255	—0.460	0.837
C_9	10.69	0.237	1.897	2.371	0.082	0.798	—0.689
C_8	11.95	0.265	2.121	2.651	—0.094	0.725	—0.583
C_7	13.21	0.293	2.344	2.930	—0.267	—0.557	0.905
C_6	14.47	0.321	2.567	3.209	—0.431	—0.913	0.255
C_5	15.73	0.349	2.791	3.489	—0.583	0.255	—0.998
C_4	16.99	0.377	3.014	3.768	—0.716	0.996	0.113
C_3	18.25	0.405	3.238	4.048	—0.827	0.075	0.955
C_2	19.51	0.433	3.462	4.327	—0.913	—0.972	—0.465
C_1	20.77	0.461	3.686	4.607	—0.970	—0.391	—0.782
O_1	21.26	0.472	3.772	4.715	—0.985	0.138	—0.218
O_2	21.40	0.475	3.798	4.747	—0.988	0.297	—0.019
					—2.044		
			$F_{(001)}/f = \Sigma \cos 2\pi \cdot 1z =$				
			$F_{(008)}/f = \Sigma \cos 2\pi \cdot 8z =$			—1.063	
			$F_{(00.10)}/f = \Sigma \cos 2\pi \cdot 10z =$				—1.526

* We thank A. Niggli for supplying the data of this table.

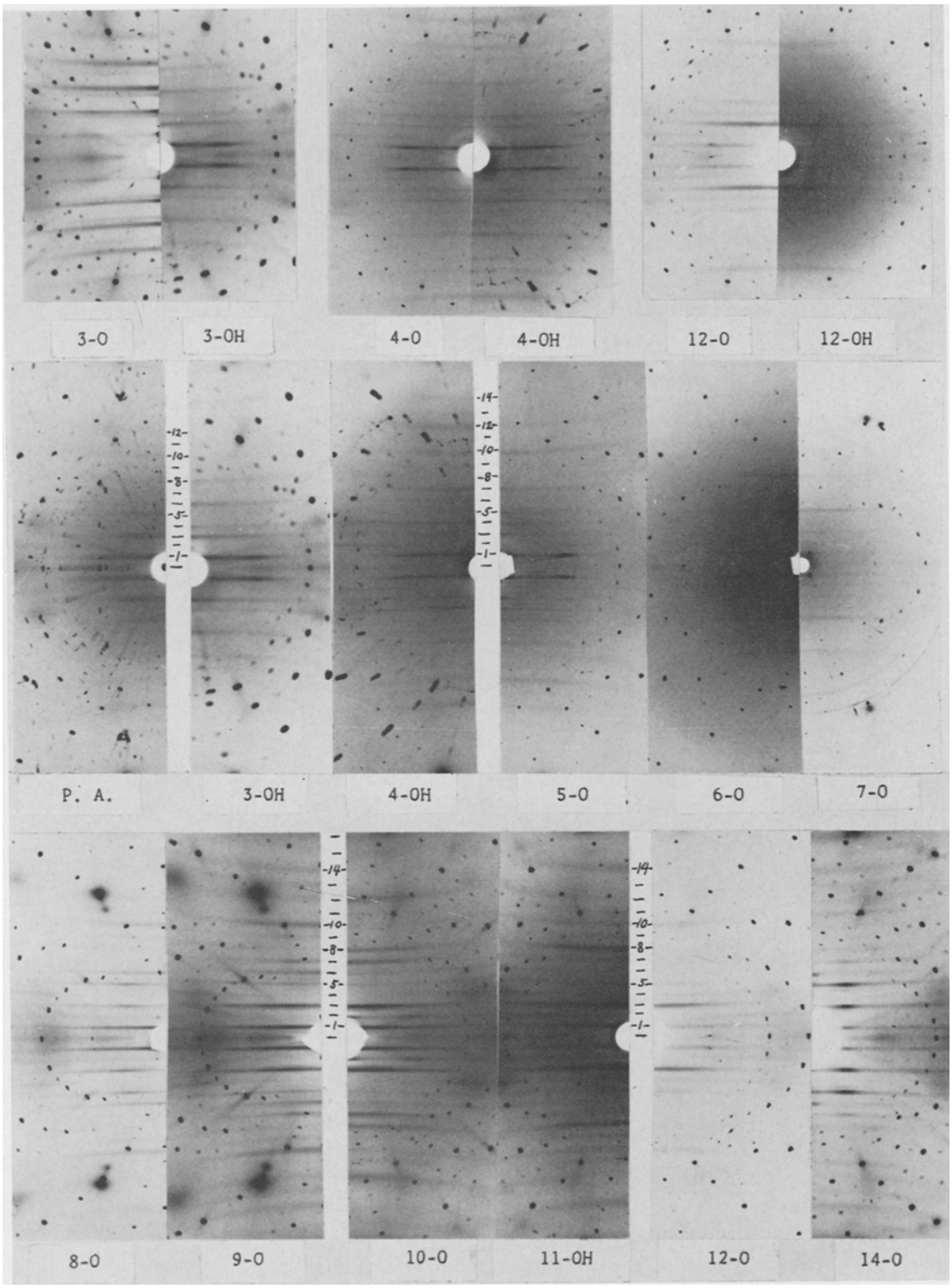
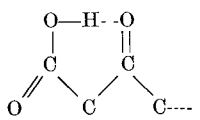


FIG. 13. Central parts of photographs of palmitic acid (p.a.) and of n keto (n-O) and of n hydroxy (n-OH) substituted palmitic acids where n indicates the position of substitution. Note that there is practically no difference in intensities between n-O and n-OH substitutions except in the case of n = 3, where the keto acid is adducted as a monomer. This is due most probably to intramolecular H-bonding to form the structure



rather than intermolecular H-bonding to form the normal carboxyl dimer structure. A series of substitutions from n = 3 to n = 14 (except n = 13) is given to show the differences in the intensities of the 00*l* lines. The *l* numbers (order numbers) range from 1 to at least 14 as indicated in the numbers between the photographs. In the case of 9- and 10-keto substitutions note that the intensity relations of the lines are drastically different for different substituted positions, especially for the lines with high *l* values. An example of intensity calculations for the lines 001, 008 and 00.10 is given in the text and in Tables VI and VII for palmitic acid and its 8- and 9-keto derivatives. In some cases, especially apparent in 3-O and 14-O a faint line accompanies each darker line. These lines are due to Kβ x-radiation whereas the darker lines are due to the Kα radiation of Cu. Kβ radiation can be removed by the use of a nickel filter according to standard practice. Only lines due to Kα radiation should be compared.

TABLE VII

Relative Intensities of the 1st, 8th and 10th Orders of Diffraction of the Continuous Layer Lines of Palmitic Acid and Its 9- and 10-Keto Derivatives [a]

	F_{001}/f	F_{008}/f	$F_{00.10}/f$	F^2_{001}/f^2	F^2_{008}/f^2	$F^2_{00.10}/f^2$	Relative Intensities		
							001	008	00.10
p.a	—2.044	—1.063	—1.526	4.18	1.13	2.33	151	3.5	4.7
9-keto-p.a	—1.962	—0.265	—2.251	3.85	0.07	5.07	139	0.02	10.1
10-keto-p.a	—1.789	—1.523	—0.725	3.20	2.32	0.53	116	7.2	1.1

[a] Compare with Figure 13.

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Acid-Treated Florisil as an Adsorbent for Column Chromatography

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Abstract

Acid-treated Florisil, prepared by the action of hot concentrated hydrochloric acid on Florisil, was found to be a useful adsorbent for separation of phospholipids and other complex lipids by column chromatography. This material gave separations similar to those obtained with commercial silicic acid but its coarse mesh size simplified the technical operations and permitted faster flow rates. Its use is illustrated by separations of model compounds and of lipids extracted from liver and brain.

Introduction

Florisil, a commercially-prepared magnesia silica gel, has been used successfully in our laboratory for several years as an adsorbent for the separation of different classes of neutral lipids by column chromatography (3). The order of elution of neutral lipid classes from Florisil columns is the same as that observed with columns of silicic acid, but Florisil has the advantage of a coarse mesh size which makes for easier handling and more rapid flow rates. In spite of its coarse mesh size, Florisil seems to have as much adsorptive surface as the commonly used fine mesh preparations of silicic acid, and lipid loads of 10 mg or more per gram of adsorbent may be readily separated without evidence of overloading.

Although Florisil proved very satisfactory for the separation of neutral lipid classes, it could not be used to separate and recover phospholipids satisfactorily. Only part of the phospholipid was recovered by eluting the columns with methanol and there seemed to be no clean separation of different phospholipid classes (3). Rouser et al. (18), working with beef brain lipids, also reported difficulties due to trailing of peaks. They found that large volumes of solvents were required for elution of phospholipids and some of the adsorbent (magnesium silicate) was eluted along with the lipids.

Free fatty acids, like phospholipids, are adsorbed more strongly on Florisil columns than on silicic acid columns, but it is possible to recover free fatty acids quantitatively by including acetic acid in the eluting solvent (3). This is a further advantage of Florisil over silicic acid, since the free fatty acid and triglycer-