These results with form I and form II of methylprednisolone demonstrate that significant differences in solubilities may exist among polymorphs. In the present example the unstable form, II, exhibits a solubility the order of 80% greater than that for the more stable form, I, at room temperature. In order to obtain an idea as to the order of magnitude of the solubility of amorphous methylprednisolone, exploratory studies[2] involving cloudpoint determinations were carried out. The results of these are presented in Fig. 2 (darkened circles). These "solubility" values represent the concentrations of the steroid in aqueous solutions necessary for rapid appearance of turbidity when a concentrated dimethylformamide solution of the steroid was added dropwise to water. If it can be assumed that appreciable supersaturation is unnecessary for nucleation of the supercooled liquid phase, it is reasonable to presume that these values represent a lower limit for the solubility of the supercooled liquid or the amorphous solid. It is noteworthy that these correspond to almost 20 times the solubility of form I. It is hoped that future studies on this problem would lead to preparable crystal forms exhibiting such large differences in solubilities.

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X-Ray and Crystallographic Applications in Pharmaceutical Research IV

Modified Procedures for Molecular Weight Determinations

By JOHN W. SHELL†

The modified procedures described in this paper for the determination of molecular weights of crystalline organic compounds differ from conventional procedures in the replacement of film recording by proportional counter recording and by the use of fractional-cell, rather than true unit-cell volumes. These procedures allow the routine determination of molecular weights with relatively small investments of time, yet with errors usually no greater than one hydrogen atom. The general method is presented and detailed procedures for lower symmetry crystal systems, in which most organic compounds fall, are described. The procedures are illustrated by specific examples.

FOR THE precise determination of molecular weights of crystalline compounds, the X-ray diffraction method has long been the method of choice. If one invests sufficient time and all the necessary care, the accuracy of this method need be limited only by errors due to crystal imperfections, which are often of the order of 0.04% if due to void spaces and often less if due in part to inclusions.

The strong relationship between time investment and accuracy appears, for practical purposes, to have a point of diminishing returns. Experience has shown that somewhat less than absolute, but yet fairly accurate determinations can be made with reasonable time investments.

In these laboratories an attempt has been made to develop procedures which assure an acceptable level of accuracy (the error usually approximating one hydrogen atom) with a minimum investment of operator time. This has required differing procedures for crystals of different symmetry, as noted below. It has also required the replacement of film techniques with direct recorder techniques.

GENERAL METHOD

The feasibility of the X-ray method for molecular weight determinations derives from the fact that an exact and determinable numbe; of molecules occupies a *unit cell* which, by three-dimensional repetition, generates the space lattice of the crystal. As the repeat spacings are of the same order of magnitude as X-ray wavelengths, diffraction of X-rays is possible and oriented diffraction procedures permit the measurement of the unit cell volume. An experimentally determined unit-cell density (crystal density) permits then the simple calculation of the unit-cell weight. This is either the molecular weight or some

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multiple of it, depending upon the number of molecules per cell. The number of molecules per cell is determinable.

The conventional approach to the measurement of the unit-cell volume has been by the use of X-ray film cameras and oriented crystal procedures producing values for the true cell edge lengths and the angular relationship between the true cell edges. A modified approach is by use of proportional counter detection and the measurement of fractional cells, rather than true cells, resulting in a more rapid accumulation of accurate information.

In the sections which follow, two crystal density determination methods are described, followed by modified procedures for the determination of unit-cell volumes for the various crystal symmetries encountered most often with organic compounds. The methods are illustrated by specific examples.

Determination of the Crystal Density

Two methods of crystal density determination have been found to be highly reliable. The first, which is more rapid, is less accurate and tends to limit the molecular weight determination to the density error, usually about 2×10^{-3} Gm./ml. The second method requires more time but allows the accuracy of the molecular weight determination to be limited to either that of the X-ray measurements or to the crystal imperfections.

Method 1.—This less accurate, but more rapid method is an adaptation of the flotation method of Bernal and Crowfoot (1), and consists essentially of immersing a few crystals in a light liquid, followed by addition of a miscible heavy liquid until the crystals tend to rise in the mixture. At this point the heavy liquid is added dropwise to the mixture with stirring. Each addition is followed by a short period of centrifugation. This is continued until complete dispersion is maintained even after prolonged centrifugation. The temperature of the suspension is noted, using the same thermometer which is later to be used as part of a standard pycnometer, and the suspension cooled to a few degrees below this temperature. The suspension is then used to fill the pycnometer and the volume adjusted when the temperature reaches the original suspension temperature. The density is then computed from the weight of the known volume.

The centrifuge should be of the clinical type, which permits the tubes to swing to a horizontal position. A convenient volume to use is 10 to 12 ml. so that standard centrifuge tubes and a 10-ml. pycnometer may be used.

When only a few crystals are available, it is convenient to do all mixing in a small (25-ml.) beaker, adding each new liquid mixture to the few crystals in a 3-ml. test tube for centrifuging. If, in the early stages of the determination, density equilibration is approached by adding the heavy liquid to the light liquid, centrifugation after each addition will pack the few crystals to the bottom of the tube and the liquid can be poured into the breaker for the next addition and mixing. This confines the crystals to the small volume of the tube and conserves them even during a large number of liquid additions and a large total volume increase.

The liquids used for flotation must obviously be nonsolvents for the crystals of interest. Both an aqueous system and a series of organic liquids have been used with good success, depending upon the solubility of the crystals. The aqueous system is comprised of various concentrations of zinc chloride which allow increases in density from 1.0 up to about 2.0 and covers the density range of most organic compounds. The addition of a slight trace of Aerosol OT, or other wetting agent, to aqueous flotation liquids has been found to be advantageous. The organic series comprises the miscible liquids benzene (d = 0.879), α-chloronaphthalene (d = 1.194), α-bromonaphthalene (d = 1.487), s-tetrabromoethane (d = 2.964), and methylene iodide (d = 3.3). With either flotation system it has been found helpful to place the container with the lighter liquid and immersed crystals into some evacuable chamber so that the crystals may be freed from occluded air within a few minutes at reduced pressure.

During centrifugation, the flotation suspension undergoes a temperature rise; this is the source of the chief error of this method. For this reason, short centrifugation time and larger volumes are advantageous. A crystal density may be determined by this method in about 1 hour.

Method 2.—The most precise crystal density determination method is that of Straumanis. Since it has been previously described (2), it will receive only brief mention here.

Use is made of a special tube-shaped pycnometer constricted at the neck with a calibration mark in the area of constriction. A flotation liquid nearly equal in density to that of the suspended crystal is added past the mark and the filled pycnometer immersed in a water bath adapted for temperature control. The temperature is adjusted until suspension is achieved, at which time the volume is adjusted to the mark and the pycnometer and contents weighed. Knowing by previous calibration the pycnometer volume over a range of temperatures, the density of the crystal is calculated. The use of a cathetometer to observe the suspended crystals in the final stages of equilibration has been found advantageous. The accuracy of the method depends upon the difference in cubical expansion of the liquid and the crystal, which is usually quite large. The accuracy reported by Straumanis (2) was at least 3×10^{-4} Gm./ml. The time required for a determination is from 2 to 3 hours.

Measurement of the Unit Cell Volume and Calculation of the Molecular Weight

Many alloys, minerals, and other inorganic compounds crystallize in high symmetry systems. The measurement of their unit-cell dimensions can often be made directly from easily obtained powder-diffraction patterns. The organic compounds of pharmaceutical interest, however, almost invariably crystallize in lower symmetry systems and their powder-diffraction patterns are too complicated for the direct determination of unit cell dimensions. This is always true for triclinic and monoclinic crystals; indirect graphical methods have been extended with with some success, particularly to orthorhombic powder patterns, but only when each powder line is known to a high degree of accuracy, and accompanied by an extremely large investment of time. It has, therefore, become standard procedure to utilize single-crystal studies in place of the more easily acquired, but less meaningful, powder patterns.

Several types of single-crystal X-ray cameras are

well known. Most of them are designed for use in total structure determination, only the first step of which involves unit-cell measurements. Most all of these cameras use film for the detection of diffracted X-rays. The recently developed General Electric single crystal orienter, however, utilizes direct recording by a proportional or scintillation counter instead of film. Its use permits accurate measurements with a saving of much of the time otherwise required for the exposure, developing, drying, and measuring of the film for determination of each dimension. Moreover, unlike the procedures required in the use of most film cameras, all measurements may be made from a single mounting of the crystal. The attachment, mounted on a G. E. XRD-5 diffractometer, is shown in Fig. 1.

For unit-cell measurements, a single crystal is selected on the basis of size, shape and the freedom from small, adhering crystals. A polarizing microscope is ideal for the purpose of crystal selection. Crystal habit varies considerably within most crops of organic crystals. It is often convenient to select a crystal with an elongated prism axis. Compared to the optimum size for inorganic crystals (about 0.2 mm.), the low mass absorption coefficients of elements comprising organic compounds permits use of fairly large crystals (up to 1 mm., if properly positioned).

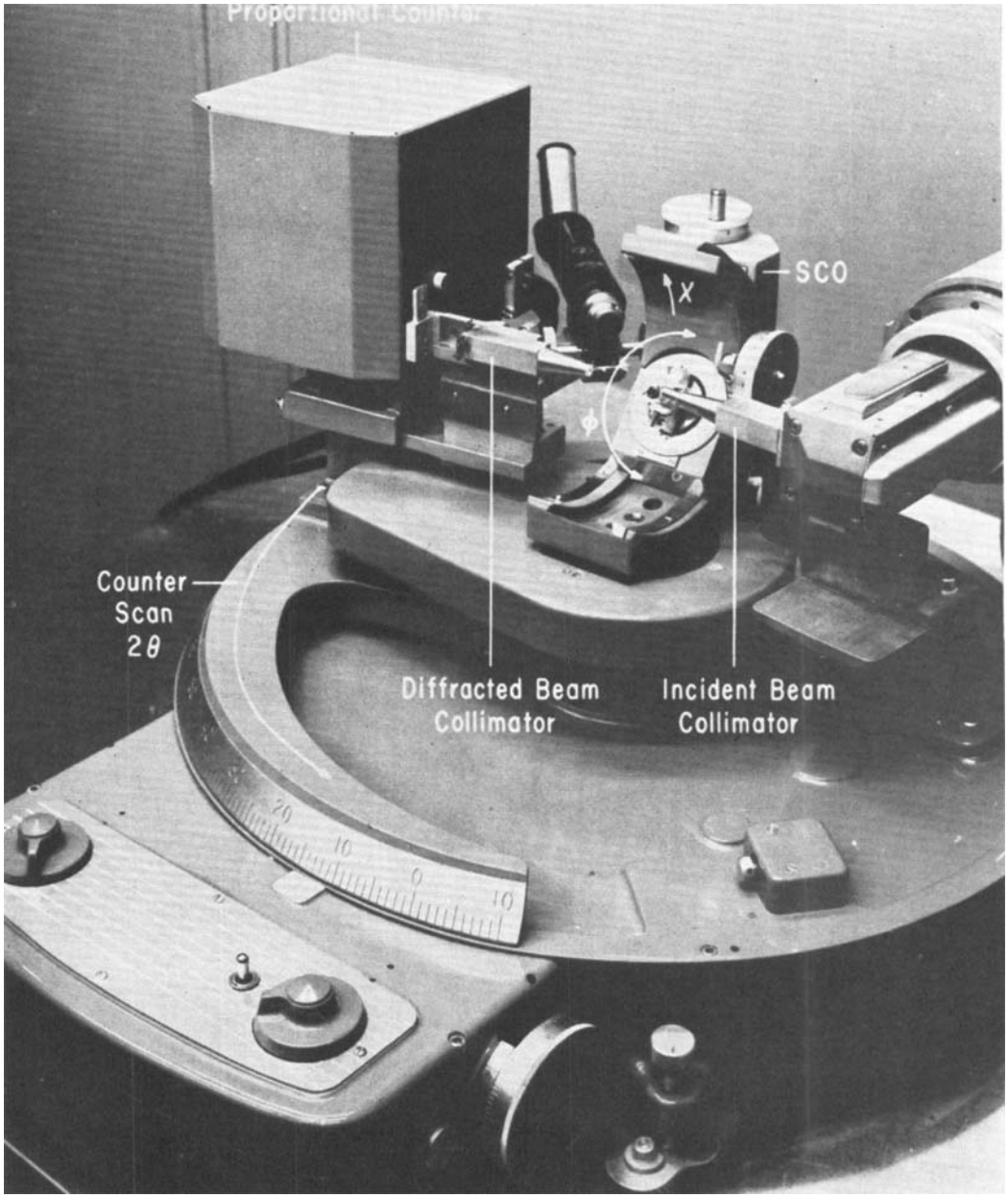

Fig. 1.—X-ray diffractometer and single-crystal orienter (SCO). Crystal rotation directions ϕ and χ are shown (radiation shields removed).

With the aid of a microscope, the crystal is cemented to the end of a fine glass fiber in such a manner that the fiber is approximately coincident with one of the crystallographic axes. The fiber is then attached to the goniometer head of the single crystal orienter (SCO) by the use of beeswax, plasticine, or other suitable medium. With the aid of the microscope shown in Fig. 1, the goniometer head permits orientation of the crystal at a point in space such that there is no departure of the crystal from this point, regardless of rotation about the various SCO axes. The principal SCO axes are phi (ϕ), about which the originally chosen crystal axis may be rotated, and chi (χ), about which this crystal axis may be inclined. The ϕ axis is rotatable through 360° and calibrated to 0.02°. The χ axis may be rotated from $-10°$ to $+100°$ and is calibrated to 0.01°. The ϕ axis is vertical when χ is set at 0°. All orientations of the crystal with respect to the X-ray beam are possible from a single mounting of the crystal. From a properly oriented crystal, the diffractometer measures the Bragg angle, 2θ, directly.

As the proportional counter shown in Fig. 1 scans in a counterclockwise direction, the SCO also rotates in this direction, but at half the angular velocity of the counter. The Bragg equation, $N\lambda = 2d \sin \theta$, relates the X-ray wavelength (λ), the diffraction angle between the diffracted and projected incident beams (2θ), and the crystal interplanar spacings (d), all for various orders of diffraction (N). Thus, when a diffraction "signal" is found at a certain 2θ position, solution of the Bragg equation gives the spacing within the crystal of parallel planes which are normal to the bisector of the angle between the incident and diffracted beams.

Since most pharmaceutically important compounds are organic, and most crystalline organic compounds are of either orthorhombic, monoclinic, or triclinic symmetry, these lower symmetry systems will be considered in detail. The higher symmetry systems, isometric, tetragonal, and hexagonal, can be treated as special, simplified cases of the lower symmetry systems described. For instance, a tetragonal crystal may be treated as an orthorhombic crystal which has two of its axes equal in length.

A. Crystals of Orthorhombic Symmetry.—Orthorhombic crystals are referred to three mutually perpendicular, unequal axes. The volume of an orthorhombic unit cell is simply the product of the three axial lengths or, in other words, the product of the dimensions of the repeat lattice in each of the three perpendicular directions.

The orthorhombic cell volume is measured by mounting the crystal with one of the crystallographic axes vertical, and coincident with ϕ, while $\chi = 0°$. With ϕ rotated until a major crystal face is parallel to the X-ray beam when $2\theta = 0°$, a scan of 2θ will generally give a first diffraction signal. Solution of the Bragg equation for the 2θ value thus obtained gives one of the cell dimensions or some submultiple of it. Repeating this operation after a rotation of 90° in ϕ produces the second cell dimension. The third cell dimension is obtained from a 2θ scan following a 90° rotation of χ. Orthorhombic axes are designated such that $c<a<b$.

The recording of 2θ data for each signal should be made only after the signal is maximized by slight adjustments of all three variables, χ, ϕ, and 2θ. Further, it should be noted that each diffraction position of the crystal may give rise to several signals at different 2θ values, each representing a different diffraction order. As certain errors diminish with increasing values of θ, higher order diffractions are advantageously used for the cell-dimension determination. Finally, this procedure sometimes results in a cell-dimension value which is one-half that of the true cell dimension, due to certain diffraction extinctions. A fractional, rather than true cell volume is thus obtained. For the purpose of molecular weight determination this is of no consequence as the error will result in only a different whole number value for the number of molecules determined per unit cell and not in a different value for the molecular weight itself.

When the crystal density and the unit-cell axes, a, b, c, (or possibly semiaxes) have been determined, the cell volume ($v = abc$ for orthorhombic crystals) and the molecular weight, M, of the compound are calculated (3)

$$M = \frac{dv}{1.6604\ n}$$

where d = density, v = cell volume, and, n = number of molecules per cell.

Although more involved methods exist for determining n, the number of molecules per cell, the simplest method which is invariably applicable consists of substituting for M in the above equation an approximate molecular weight value obtained from chemical evidence and solving for n. Knowing that the only possibilities for n in this crystal system are 1, 2, 4, 8, etc., the proper choice becomes obvious and the exact value of n may be used to calculate M.

Sometimes a significant gain in accuracy can be accomplished by the combined use of single-crystal data and powder-diffraction data. This can often be accomplished at an overall saving of time, since when this procedure is followed, the single-crystal data need not be so precise and can, therefore, be collected more rapidly.

The procedure involves rapid measurement of unit cell dimensions by the SCO operation described above and the use of these approximate values to partially index a powder diffraction pattern.[1] By a slow scan of only the three appropriate powder diffraction lines, using standard diffractometer techniques for high resolution,[2] the cell dimensions can be measured with extreme accuracy.

Examples of the results of the use of combined single-crystal and powder-diffraction data are shown

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[1] For orthorhombic crystals,

$$d = \frac{1}{\sqrt{\frac{h^2}{a^2} + \frac{k^2}{b^2} + \frac{l^2}{c^2}}}$$

This reduces greatly for the determination of d for $h00$, $0k0$ and $00l$, usually found, with organic crystals, in the first few powder lines. Occasionally $hk0$, $h0l$, or $0kl$ will have to be calculated also, if one of the first series is absent.

[2] It has been found useful to mix a small amount of sodium chloride with the unknown material before measuring the powder diffraction pattern. The 2θ positions of all measured lines may then be corrected by the measured position of a sodium chloride line, and errors due to instrumental misalignment, temperature, etc. are compensated. Sodium chloride has a d spacing (100) of 5.64009Å. at 25°.

TABLE I.—RESULTS OF THE USE OF PRECISE POWDER DIFFRACTION DATA TO REFINE APPROXIMATE SINGLE-CRYSTAL DATA FOR UNIT-CELL MEASUREMENTS

	Crystal Density	Cell Dimensions, Å.		Molecular Weight					
				Approximate			Refined		
		Approximate	Refined	Found	True	% Error	Found	True	% Error
Example 1	1.255	a = 9.30	9.50						
		b = 11.01	11.00						
		c = 5.10	5.08	394.70	401.49	1.7	401.25	401.49	0.06
Example 2	1.197	a = 8.46	8.34						
		b = 12.86	12.93						
		c = 3.99	3.99	312.94	309.39	1.1	310.18	309.39	0.26

in Table I, as applied to two different crystalline steroids of orthorhombic symmetry. Approximately 1 day was required for each example.

The modified method described here usually results in a gain in both time saved and accuracy. A comparison of these variables for the modified and the conventional film methods is presented in Table II using the orthorhombic crystal tolbutamide for an example. The molecular weight values were calculated using an experimentally determined density value of 1.245.

TABLE II.—COMPARISON OF MODIFIED METHOD AND CONVENTIONAL-FILM METHOD FOR THE ORTHORHOMBIC CRYSTAL TOLBUTAMIDE, MOL. WT. = 270.34

	Conventional Film Method (4)	Modified Method
Unit cell dimensions, Å.	a = 9.07	a = 9.116
	b = 20.14	b = 20.32
	c = 7.78	c = 7.770
Cell volume, Å.^{3}	1421.2	1439.3
Molecular weight, calculated	266.4	269.3
Per cent error in molecular weight	1.45%	0.20%
Time required for result:		
Operator hours	6	4
Total hours	17	8

B. Crystals of Monoclinic Symmetry.—Monoclinic crystals are referred to three unequal axes, two of which, a and c, intersect at an oblique angle, β, and with the b axis normal to the plane of a and c.

The SCO procedure found most useful for the rapid but accurate determination of a monoclinic cell, which again is not necessarily the true unit cell, is as follows.

The crystal is mounted so that the ϕ rotation is about the b crystallographic axis. The procedure is similar to that used for orthorhombic crystals, except that the angular measurement of β (measured in ϕ) is required. Using crystal faces as a guide for a first diffraction signal with χ at 0°, the ϕ and 2θ values are recorded and signals at other ϕ positions sought by scanning both ϕ and 2θ. Only fortuitously would a second signal be found with a ϕ setting 90° from the ϕ setting of the first signal, as would be found with orthorhombic crystals. Several signals at different settings will usually be found, and two ϕ values should be selected for use which are as close to 90° apart as possible. The difference between these ϕ settings is the β angle. The 2θ values at each ϕ setting, when converted to interplanar spacings, may be used to compute the area of a monoclinic cell in the ac plane. Rotation of χ to 90° permits determination of a 2θ value which, when converted, is the cell length along b.

Conventional procedures result in values for a, b, and c, representing true cell edge lengths, and the angle β. The SCO procedure described here measures interplanar spacings which are not necessarily the same as cell edge lengths.

Appropriate interplanar spacings are the same as cell edge lengths in orthorhombic crystals, and one set of interplanar spacings is the same as one cell length in monoclinic crystals (the 90° χ position gives true b). Figure 2 shows a planar section (normal to b) through a monoclinic crystal; a and c are true cell edge lengths; a' and c' are the values obtained from the SCO procedure. The area of the section is $ac \sin \beta$, or $a'c'/\sin \beta$ and the cell volume is $abc \sin \beta$ as conventionally determined, or $a'bc'/\sin \beta$ as determined by the SCO procedure.

Exemplifying the method are the results of the application to the determination of the cell size and the molecular weight of methylprednisolone. This compound crystallizes under normal conditions in the monoclinic system with the true unit-cell constants shown in Table III.

TABLE III.—TRUE UNIT-CELL CONSTANTS FOR THE MONOCLINIC CRYSTAL METHYLPREDNISOLONE

a = 21.78 Å.	Cell volume—1979 Å.^{3}
b = 9.183	n = 4
c = 10.88	Density—1.2544
β = 65° 26.1′	

With the crystal mounted so that the ϕ rotation was about the b axis, and with χ = 0°, signals were found at ϕ settings 65° 26.1′ apart. The 2θ values for these settings gave interplanar spacing values of

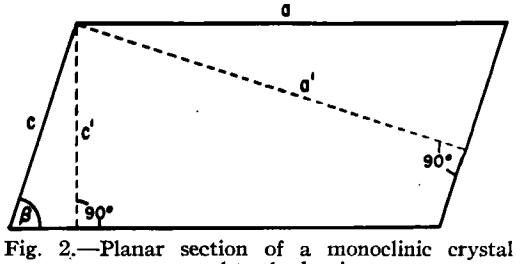

Fig. 2.—Planar section of a monoclinic crystal normal to the b axis.

9.904 Å. and 9.895 Å. Following rotation of χ to 90°, conversion of the measured 2θ value gave a result of 4.591 Å. These values gave a calculated cell volume of 494.69 Å.^{3} which, together with the measured density value of 1.2544, allowed the calculation of the molecular weight, 373.8. (True molecular weight, 374.46.)

The volume of the subcell measured by the SCO procedure is one-fourth that of the true unit cell, as two of the measurements were of hemiaxes. Again, the fact that the modified method measures a fractional cell is of no consequence when used for molecular weight determinations. In fact, the method quite often gives the molecular weight directly, as in the example just cited, rather than some multiple of it.

As with the determination of orthorhombic cell volumes, a gain in accuracy can sometimes be achieved with monoclinic cells by combining SCO data and at least partial powder-diffraction data. The probability of doing so at a saving of time is not so great as with orthorhombic crystals, however, due to the greater complexity of the indexing problem. This is so when the indexing is done manually. With the aid of machine methods, however, the gain in accuracy can be made with no time loss. By programming a digital computer[3] to solve the following equation[4] for all values of h, k, and l,

$$d = \frac{1}{\sqrt{\frac{\frac{h^2}{a^2} + \frac{l^2}{c^2} - \frac{2hl}{ac}\cos\beta}{\sin^2\beta} + \frac{k^2}{b^2}}}$$

given a, b, c, and β, a list of d spacings can be produced. This list is essentially a synthetic powder pattern whose d values are of no greater accuracy than the SCO data but which is accurately indexed. Comparison with the experimental powder pattern (accurate d values, but not indexed) results in accurate indexing of the accurate d values, which may then be used to compute the cell volume.

C. Crystals of Triclinic Symmetry.—Triclinic crystals are referred to three unequal axes, all intersecting at oblique angles. Such crystals have undergone such an extreme decrease in symmetry when compared to monoclinic crystals that the cell volume calculation is highly involved. Further, measurements of three oblique angles give rise to major sources or error. When using conventional film techniques, one almost invariably gains in both time and accuracy of the cell-volume determination when time is invested in converting the organic crystal to one of higher symmetry through either a polymorphic change, solvation (the degree to be later determined), or derivativization.

Although the SCO is ideally suited for the determination of the true unit cell edge lengths and true interaxial angles, for purposes of molecular weight determinations the true cell constants need not be found. As pointed out by Bunn as early as 1945 (4), the volume of a triclinic cell is one axis length multiplied by the area of that axis projection. Using film techniques, one could measure the layer-line spacings, achieving the axis length, and theoretically, from equatorial spacings, the dimensions of the axis projection. Finding the axis projection required the determination of an oblique angle, however, as well as several reflection orders of two different dimensions, all from one row of superimposed spots. With film techniques, then, the axis projection method is still quite complicated.

The availability of the SCO allows easy application of the axis-projection method to triclinic cells. Errors are significantly reduced by the reduction in the number of required angles measured from three to one and even when the SCO method is combined with a partial film method (to measure one axis length) there is a great saving of time. As mentioned above, the SCO is capable of measuring all triclinic cell constants. Experience in these laboratories has shown, however, that the most rapid procedure with acceptable accuracy involves a combination of SCO and film techniques. This procedure is described below.

With the crystal mounted in the SCO ($\chi = 0°$) so that one crystal axis is coincident with ϕ, scans of ϕ and 2θ are made until signals corresponding to two different settings of ϕ are found. The 2θ values are converted to interplanar spacings and recorded along with the angle between the two ϕ settings. The product of these spacings, divided by the sine of this angle, is the area of the axis projection. The goniometer head, together with the undisturbed mounted crystal, can then be transferred to a film camera for a standard rotation photograph. The layer-line spacings of the rotation photograph allow the calculation by standard techniques of the axis length. The product of this length and the area of the axis projection is the volume of the cell. The cell measured in this manner is not necessarily the true unit cell but bears some whole number relation to it so that an accurate molecular weight value can be found.

In order to compare the conventional and the modified techniques, as applied to a triclinic crystal, cholesterol may be used as an example. The true unit cell constants of cholesterol (5) are shown in Table IV. The volume of a triclinic cell is $v = abc$

TABLE IV.—TRUE UNIT-CELL CONSTANTS FOR THE TRICLINIC CRYSTAL CHOLESTEROL

$a = 14.10$	$\alpha = 94.60°$
$b = 33.75$	$\beta = 90.00°$
$c = 10.46$	$\gamma = 95.72°$

$\sin\beta \sin\gamma \sin\delta$ where

$$\sin\frac{\delta}{2} = \sqrt{\sin\frac{(\alpha - \beta + \gamma)}{2}\sin\frac{(\alpha + \beta - \gamma)}{2}}$$

The calculated volume of the cholesterol unit cell is 4939.7 Å.^{3}. All six constants listed above were required to calculate this value.

Applying the modified method with the crystal mounted with c and ϕ coincident, values of 33.60 Å. and 13.97 Å. are recorded from two separate ϕ settings, which are 95°42′ apart. These correspond to the 010 and 100 reflections. After determining

[3] The author is grateful to Dr. O. S. Carpenter of The Upjohn Co. for programming and operation supervising of the Burroughs E-102 digital computer.

[4] It may be noted that for orthorhombic crystals, where $\beta = 90°$, this equation reduces to that presented in the previous section, footnote 1. The same computer program may therefore be used to index powder patterns of both orthorhombic and monoclinic symmetry.

the c axis length, 10.46 Å., from a single rotation photograph, a simple calculation gives v'

$$v' = \frac{33.60 \text{ Å.} \times 13.97 \text{ Å.} \times 10.46 \text{ Å.}}{\sin 95°42'} = 4934.2 \text{ Å.}^3$$

This value agrees well with the previously calculated v for the true unit cell, 4939.7 Å.^{3}. It should be noted that v' was obtained by a simpler procedure which is usually more rapid and, as noted above, the requirement of measuring only one, instead of three angles, usually results in a significant gain in accuracy.

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Effect of Drugs on Survival Time from Scorpion Envenomation

By LEO GREENBERG and JAMES W. INGALLS

Androctonus australis **venom at various dilutions was administered to matched groups of mice and the effects on survival time and number determined for various classes of therapeutic agents. Meperidine and other narcotics were consistently deleterious to envenomed animals as were alcohol and calcium acetylsalicylate carbamide. Neither corticosteroids nor muscle relaxants were beneficial. With tranquilizing agents, meprobamate tended to increase survival time, while reserpine markedly curtailed survival.**

In previous papers from our laboratories, we have investigated the interaction of tranquilizing drugs and various toxic agents in laboratory animals. These challenges have included curare (1), bacterial exotoxins (2–4), bacterial endotoxins (3, 4) and septicemias (2, 3). Presently, reports are in preparation on amoebiasis and trichinosis. It was of interest therefore to determine if the profound alterations in survival time evinced with tranquilizer treatment in the previous conditions could be demonstrated with arthropod venom. Moreover, it was felt that such a study might have more than academic interest since scorpion envenomation represents a major hazard to man and animals throughout much of the tropical and semitropical areas of the earth's surface. In some areas, principally North Africa, the danger from scorpions is greater than from venomous snakes (5).

It is known that the pharmacological actions of scorpion venom from a great variety of genera and species are quite similar (6) and that species widely separated by geography contain venom components in common (7). Symptoms of envenomation include muscular contractions, salivation, respiratory paralysis, and vasoconstriction. It is suggested that these indicate the existence of a substance or substances with strong parasympathetic activity acting in a similar manner to serotonin (8).

In the present study, in addition to tranquilizing drugs, other agents of real or imagined therapeutic significance in the envenomed state were included for comparison. The narcotics meperidine,[1] cocaine, codeine, and morphine were tested in light of the findings of Stahnke (9, 10) that both morphine and meperidine are undesirable therapeutic agents for use in envenomation from the Arizona scorpion, *Centruroides sculpturatus*. The analgesics calcium acetylsalicylate carbamide[2] and dextropropoxyphene hydrochloride[3] were included as possible substitutes for the narcotic agents in controlling pain. Methocarbamol[4] was tested on the basis of its reported value in cases of black widow spider bites (11), and two other muscle relaxants, carisoprodol[5] and phenyramidol hydrochloride,[6] were included for comparison. In view of the reported parasympathomimetic action of scorpion venoms, the antiparasympathetic scopolamine was included in this study. Alcohol was used in keeping with the long standing proscription against whisky in snake bite, and the barbi-

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[1] Marketed as Demerol by Winthrop Laboratories.
[2] Marketed as Calurin by Dorsey Laboratories.
[3] Marketed as Darvon by Eli Lilly and Co.
[4] Marketed as Robaxin by A. H. Robins Co., Inc
[5] Marketed as Soma by Wallace Laboratories.
[6] Marketed as Analexin by Irwin, Niesler & Co.