The difference between the two C–O bond distances, 0·01 Å, is possibly significant; however, it is far less than the difference of 0·06 Å found by Donohue (1950) in DL-alanine. This difference was interpreted by Donohue to represent the effect of the hydrogen bonds on the resonance character of the carboxyl group. Since the hydrogen bonding is essentially identical in the two structures, it now appears that its effect on the dimensions of the carboxyl group is slight.

The bond distances and angles involving the hydrogen atoms are given in Table 5. It is interesting to note that the positions of the hydrogen atoms, as determined from a difference map and refined by least-squares methods, are very nearly the same (after appropriate transformations due to the different space groups and choices of axes) as those in DL-alanine as deduced by Levy & Corey from packing considerations. As in DL-alanine, methyl carbon atoms C(3) related to one another by screw axes along *c* are separated by only 3·68 Å – considerably less than the value 4·0 Å usually taken as the diameter of a methyl group (see, *e.g.*, Pauling, 1960). Accordingly, the hydrogen atoms mesh in the manner described by Levy & Corey; the closest H–H approaches are 2·5 Å between H(5) and H(7) and 2·7 Å between H(6) and H(7).

The work described here was carried out as an undergraduate research project (HJS). We are grateful to Mr Earl Ubell for his assistance.

References

Berghuis, J., Haanappel, IJ. M., Potters, M., Loopstra, B. O., MacGillavry, C. H. & Veenendaal, A. L. (1955). *Acta Cryst.* **8**, 478.
Bernal, J. D. (1931). *Z. Kristallogr.* **78**, 363.
Busing, W. R. & Levy, H. A. (1964). *Acta Cryst.* **17**, 142.
Donohue, J. (1950). *J. Amer. Chem. Soc.* **72**, 949.
Levy, H. A. & Corey, R. B. (1941). *J. Amer. Chem. Soc.* **63**, 2095.
McWeeny, R. (1951). *Acta Cryst.* **4**, 513.
Pauling, L. (1960). *The Nature of the Chemical Bond,* 3rd ed., p. 260. Ithaca: Cornell Univ. Press.

Acta Cryst. (1966). **20**, 555

The Crystal and Molecular Structure of Anemonin, $C_{10}H_8O_4$

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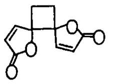
Anemonin,

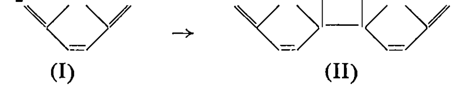


is the dimer of protoanemonin, a substance found in the plants of the crowfoot family (Ranunculaceae). While the structural formula of anemonin was fairly well established, the stereochemical relationship of the lactone rings was not. The material crystallizes in the orthorhombic system, space group *Pbca*, with eight molecules in the unit cell and cell parameters $a = 11{\cdot}65$, $b = 13{\cdot}86$, $c = 11{\cdot}07$ Å. The crystal structure analysis has established that the lactone rings are in the *trans* configuration. Furthermore, it has been shown that the cyclobutane ring is bent with a dihedral angle of 152° and that the C–C bond lengths in the cyclobutane ring have normal single-bond values near 1·54 Å.

The structure was solved by obtaining the phases directly from the structure factor magnitudes by means of the symbolic addition procedure.

Introduction

Protoanemonin (I), a disagreeable vesicant liquid, may be obtained by crushing the plant tissue of the crowfoot family (Ranunculaceae) (Heyer, 1792; Hill & Van Heyningen, 1951). It undergoes spontaneous dimerization in aqueous solution to yield crystalline anemonin (II) (Asahina & Fujita, 1920). Only one cyclodimer is formed where several different combinations seem equally possible. While the structural formula of anemonin was fairly well established, the stereochemical relationship of the lactone rings was not. It had been assumed that the lactone rings were in the *cis* configuration (Harris, 1959). The crystal structure investigation was carried out in order to establish the stereoconfiguration. A short note concerning the chemical properties and the configuration of anemonin has been published (Moriarty, Romain, Karle & Karle, 1965).



(I) (II)

Experimental

Colorless, transparent crystalline fragments of anemonin were kindly supplied by Prof. R. Moriarty of the Catholic University of America. The crystals had no recognizable faces and appeared almost glassy. Since there was a weak extinction under crossed Nicol prisms, mounting the crystals parallel to a cell edge was not very difficult. The cell parameters, determined from precession photographs using Cu $K\alpha$ radiation, are:

$$a = 11{\cdot}65 \pm 0{\cdot}02 \text{ Å},$$
$$b = 13{\cdot}86 \pm 0{\cdot}03$$
$$c = 11{\cdot}07 \pm 0{\cdot}02$$
$$\alpha = \beta = \gamma = 90°, \text{ and } Z = 8.$$

The computed density is 1·418 g.cm^{-3} while the density measured by flotation in a silver nitrate solution was 1·428 g.cm^{-3}. Systematic absences of reflections indicated that the space group is *Pbca*.

Intensity data were collected by the multiple-film, equi-inclination Weissenberg technique. Layers 0 through 8 were recorded along the *a* axis. Visual estimates were made of the intensities by comparison with a calibrated film strip. Corrections for Lorentz and polarization factors and spot size were computed, but no absorption correction was applied. The data were adjusted to an absolute scale by means of a *K* curve (Karle & Hauptman, 1953). Both the structure factor magnitudes, $|F_{\mathbf{h}}|$, and the normalized structure factor magnitudes, $|E_{\mathbf{h}}|$, were computed. For space group *Pbca*,

$$E_{\mathbf{h}}^2 = F_{\mathbf{h}}^2 / \varepsilon \sum_{j=1}^{N} f_{j\mathbf{h}}^2 \qquad (1)$$

where $\varepsilon = 2$ when $\mathbf{h}$ is $0kl$, $h0l$, or $hk0$, $\varepsilon = 1$ otherwise, $f_{j\mathbf{h}}$ is the atomic scattering factor for the jth atom, N is the number of atoms in the unit cell and the $F_{\mathbf{h}}^2$ have been corrected by means of the *K* curve for thermal motion and placed on an absolute scale.

The statistical averages and the distribution of the 1436 reflections are shown in Table 1. The unobserved reflections were assigned zero intensity. The experimental values correspond to a crystal with a center of symmetry.

Phase determination

The symbolic addition procedure has been used to determine the phases directly from the structure factor magnitudes for a large number of centrosymmetric crystals belonging to various space groups. Several references to its description and application are Karle & Karle (1963, 1964, 1965) and Karle & Dragonette (1965). A historical review of methods for determining phases directly from the structure factor magnitudes may be found in a review article by Karle (1964).

Table 1. *Statistical averages and distributions of* $|E|$

	Experimental	Centro-symmetric	Noncentro-symmetric
$\langle\|E\|\rangle$	0·786	0·798	0·886
$\langle\|E^2-1\|\rangle$	0·941	0·968	0·736
$\langle\|E\|^2\rangle$	0·991	1·000	1·000
$\|E\| > 3$	0·1 %	0·3 %	0·01 %
$\|E\| > 2$	4·5 %	5·0 %	1·8 %
$\|E\| > 1$	27·8 %	32·0 %	36·8 %

Table 2. *Assignments for the application of* Σ_2

h k l	$\|E\|$	Phase
7,11,8	2·51	+
3, 7,9	2·81	+
1,16,5	3·62	+
2,14,6	2·40	*a*
2, 4,4	2·28	*b*
2, 7,7	2·48	*c*

In the present investigation, the origin of the unit cell was specified by assigning positive phases to three linearly independent reflections having large $|E|$ values. Letter phases *a*, *b*, and *c* were assigned one at a time, as required, to three additional vectors also having large $|E|$ values in order to facilitate the application of the Σ_2 relationship which is

$$sE_{\mathbf{h}} \sim s \sum_{\mathbf{k}} E_{\mathbf{k}} E_{\mathbf{h}-\mathbf{k}} \qquad (2)$$

where *s* means 'sign of'. Using the six assignments, listed in Table 2, with the Σ_2 relationship, 137 phases for $|E| > 1{\cdot}5$ were obtained in terms of the unknowns *a*, *b*, and *c*.

As the phase determination progressed, many terms entered into the sum in (2). Some of these terms indicated relationships between the unknown symbols. In this investigation, there was only one possible assignment for the unknowns *a*, *b*, and *c*, that is $a = -$, $b = -$, and $c = +$. Any other assignment would have resulted in very many discrepancies among the contributors to Σ_2. The small number of discrepancies which did occur in Σ_2 among individual terms forming a sum for a particular reflection $\mathbf{h}$, using $a = b = -$ and $c = +$, was consistent with the number expected from the probability expression that the sign indicated by (2) be correct. For individual terms, the probability that the sign of $E_{\mathbf{h}}$ is plus is

$$P_+(E_{\mathbf{h}}) = \tfrac{1}{2} + \tfrac{1}{2} \tanh \frac{\sigma_3 |E_{\mathbf{h}}| E_{\mathbf{k}} E_{\mathbf{h}-\mathbf{k}}}{\sigma_2^{3/2}} \qquad (3)$$

where $\sigma_n = \sum_{j=1}^{N} Z_j^n$, N is the number of atoms in the unit cell, and Z_j is the atomic number of the jth atom. When there are several contributors to the sign of $E_{\mathbf{h}}$, equation (3) is altered by summing the argument of the tanh over $\mathbf{k}$.

An *E* map (Fourier map with *E* rather than *F* values for coefficients) (Karle, Hauptman, Karle & Wing, 1958) was computed with the 137 reflections for which phases had been determined by Σ_2. All fourteen atoms in the asymmetric unit were well resolved. Sections from this map are illustrated in Fig. 1.

The refinement

Coordinates for the fourteen heavy atoms as read from the E map were subjected to a least-squares refinement using a modification of the ORFLS program (Busing, Martin & Levy, 1962). The function which was minimized was $\Sigma(F_o-F_c)^2$. Individual scale factors for each layer were refined. Several cycles of isotropic refinement and then anisotrotropic refinement resulted in an R index of 14·5%. A difference map (Fig. 2) computed at this stage revealed the positions of the eight hydrogen atoms. Inclusion of the hydrogen atoms as fixed parameters in the least-squares refinement reduced R to 13·0%*. The irregular shape of the crystal used for collecting the intensity data has probably introduced errors which preclude improving the refinement to a lower R value.

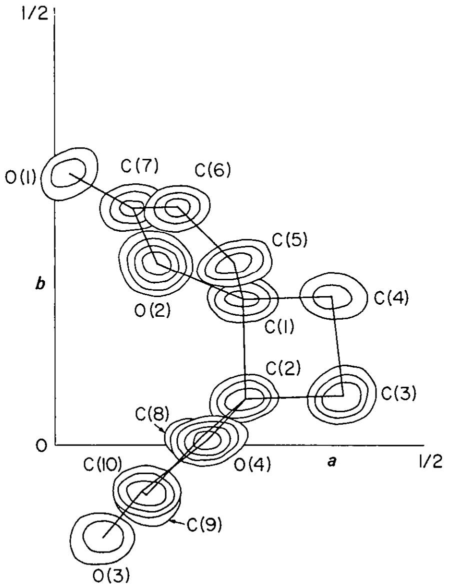


Fig. 1. Sections from a three-dimensional E map projected on (001). The map was computed from 137 independent reflections whose phases were determined directly from the structure factor amplitudes. The contours are equally spaced on an arbitrary scale.

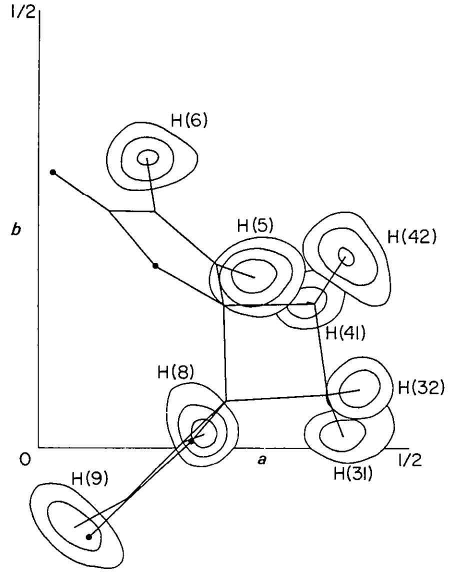


Fig. 2. Difference map illustrating the electron density for the hydrogen atoms. The contours are spaced at 0·25 e.Å^{-3}, beginning with the 0·25 e.Å^{-3} level.

Table 3. *Fractional coordinates* and thermal parameters for anemonin*

The thermal parameters are defined by $T=\exp\{-(\beta_{11}h^2+\beta_{22}k^2+\beta_{33}l^2+2\beta_{12}hk+2\beta_{13}hl+2\beta_{23}kl)$.
Each β is multiplied by 10^4.

Atom	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
C(1)	0·2505	0·1645	0·3967	16	31	29	7	−6	−1
C(2)	0·2559	0·0535	0·3991	19	31	17	0	0	2
C(3)	0·3878	0·0622	0·3898	19	45	88	13	4	3
C(4)	0·3714	0·1649	0·3423	36	46	63	3	10	9
C(5)	0·2403	0·2104	0·5195	32	33	21	3	−19	−3
C(6)	0·1569	0·2708	0·5222	48	34	30	−2	−10	−10
C(7)	0·0969	0·2722	0·3998	17	31	38	2	2	6
C(8)	0·1935	0·0046	0·2945	65	34	15	3	−15	−10
C(9)	0·1214	−0·0591	0·3360	79	36	32	−5	−33	−5
C(10)	0·1261	−0·0570	0·4714	60	28	40	6	−3	2
O(1)	0·0188	0·3176	0·3630	45	42	49	22	−19	7
O(2)	0·1580	0·2072	0·3297	19	33	16	11	−10	4
O(3)	0·0700	−0·1028	0·5425	67	46	65	−8	9	21
O(4)	0·2068	0·0078	0·5041	35	31	11	−6	−7	−5
Standard deviation									
C	0·0010	0·0006	0·0007	11	5	6	5	6	4
O	0·0006	0·0004	0·0005	6	4	4	4	5	3

* Coordinates are so chosen that they may be substituted directly into equations (3) and (4) for the planes through the lactone rings.

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* Observed and calculated structure factors have been deposited as Document number 8443 with the ADI Auxiliary Publications Project, Photoduplication Service, Library of Congress. Washington, D.C. A copy may be secured by citing the Document number and by remitting $2·50 for photoprints, or $1·75 for 35 mm microfilm. Advance payment is required. Make checks or money orders payable to : Chief, Photoduplication Service, Library of Congress.

The parameters for the heavy atoms are given in Table 3 and the hydrogen coordinates as read from the difference map are shown in Table 4. Sections from an electron density map computed with these parameters are shown in Fig. 3. They are projected on the (100) plane.

The structure

The feature of particular interest in this investigation is the orientation of the two lactone rings which have been found to occur in a *trans* configuration with respect to each other. Another interesting feature is the bent configuration of the cyclobutane ring. The molecule has an approximate twofold axis joining the halfway points between atoms C(1), C(2) and between atoms C(3), C(4),

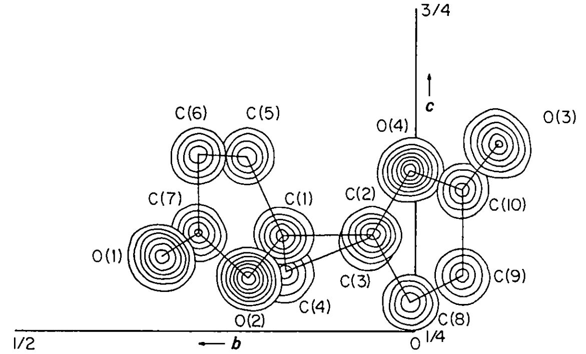

Fig. 3. Sections from the final electron density map projected on (100). The contours are spaced at 1 e.Å^{-3} beginning with the 1 e.Å^{-3} level.

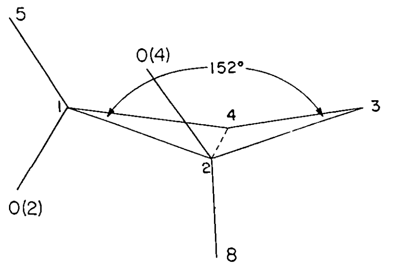

Fig. 4. Dihedral angle in the cyclobutane ring.

Table 4. *Approximate coordinates for the hydrogen atoms*

Atom	x	y	z
H(31)	0·412	0·012	0·338
H(32)	0·433	0·067	0·467
H(41)	0·362	0·166	0·277
H(42)	0·413	0·220	0·367
H(5)	0·292	0·195	0·575
H(6)	0·150	0·333	0·560
H(8)	0·225	0·017	0·205
H(9)	0·050	−0·092	0·300

The two lactone rings are essentially planar. The equation for an average* plane through the atoms C(1)C(5)C(6)C(7)O(1)O(2) is

$$0·61040ax + 0·73535by - 0·29439cz = 2·1676 \quad (3)$$

and for the atoms C(2)C(8)C(9)C(10)O(3)O(4) the equation is

$$0·70180ax - 0·71217by - 0·01770cz = 1·4945 \quad (4)$$

where a, b, and c are the cell lengths and the value on the right hand side is the plane-to-origin distance in Å. The r.m.s. deviation of the six atoms from the first plane is 0·009 Å and the r.m.s. deviation of the six atoms from the second plane is 0·014 Å. The dihedral angles formed between the planes of the lactone rings and the planes formed by C(4)C(1)C(2) and C(1)C(2)–C(3), respectively, of the cyclobutane ring are 89·5°.

The dihedral angle in the cyclobutane ring in anemonin is 152° (Fig. 4). Confirmation of the dihedral angle has been obtained from a nuclear magnetic-resonance spectrum (Lustig & Moriarty, 1965). Similar puckering of the cyclobutane ring had been observed in C_4H_8 (Dunitz & Schomaker, 1952), C_4F_8 (Lemaire & Livingston, 1952) and C_4Cl_8 (Owen & Hoard, 1951) where the dihedral angles were of the order of 160°. However, a

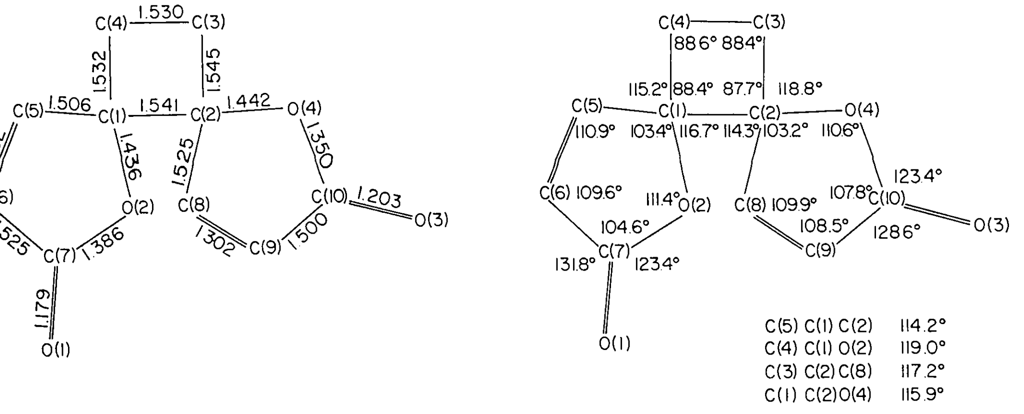

Fig. 5. Bond lengths and angles in anemonin. The standard deviation for the bond lengths ranges from 0·010 to 0·017 Å and the standard deviation for the angles is of the order of 0·8°.

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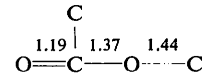
* Not obtained by least-squares.

planar ring had been found in 1,2,3,4-tetraphenylcyclobutane (Dunitz, 1949) and in the photodimer of cyclopentanone (Margulis, 1965). The C–C bond lengths in the cyclobutane ring in this investigation range from 1·530 to 1·545 Å, values which are considered normal for C–C single bonds. In each of the other five determinations mentioned above, the C–C bond lengths in the four-membered rings ranged from 1·54 to 1·60 Å.

The bond lengths and angles in the lactone rings are illustrated in Fig. 5. The values for the C–C and C=C bonds fall within the range usually observed for these types of bonds. In the lactone group, the averages of the two sets of values are



$$\overset{\overset{C}{|\,1.19}}{} \; O\overset{1.19}{=\!=}\overset{\overset{C}{|}}{C}\overset{1.37}{-\!-}O\overset{1.44}{-\,-}C$$

where the bond length for the C–O adjacent to the C=O is near that found for carboxylic acid groups, whereas the value for the other C–O bond length is near that found for paraffinic –C–OH. Large differences in length between the two C–O bonds in a lactone ring also have been observed in himbacine hydrobromide (Fridrichsons & Mathieson, 1962), bromodilactone from jacobine (Mathieson & Taylor, 1963) and *p*-bromobenzoate-glaucorubin (Kartha & Haas, 1964).

The contents of a unit cell are shown in Fig. 6. The closest approaches between atoms of different molecules are between O(3) and O(3′), related by a center of symmetry, at 3·41 Å, between O(2) and C(6″) at 3·42 Å and between C(4) and O(1‴) at 3·55 Å (occurring between molecules related by glide-planes), and between C(3) and O(1^{iv}) at 3·55 Å (occurring between molecules related by a screw axis). All other intermolecular distances are greater than 3·6 Å.

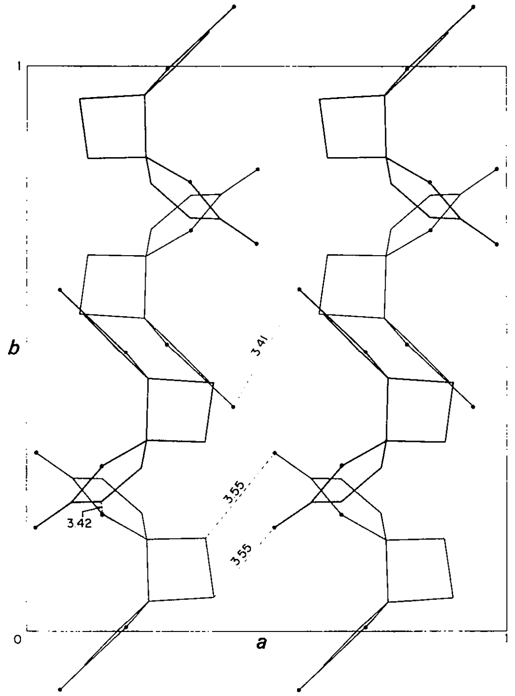


Fig. 6. Contents of a unit cell and nearest approaches between atoms of different molecules.

The molecule is rather rigid. Only atoms C(3) and C(4), which are not part of the lactone rings, have sizable vibrations along the *c* axis. Any detailed analysis of the vibrational parameters is not warranted in view of the odd shape of the crystals and lack of experimental correlation of the layers. The scale factors for the individual layers obtained from the isotropic refinement were held constant for the anisotropic refinement. The values of the scale factors did not vary by more than 10% from each other.

We are grateful to Prof. R. Moriarty for suggesting this investigation and supplying us with the crystalline material and to Dr Donald Mitchell for performing all the calculations on the IBM 7030 (STRETCH).

References

Asahina, Y. & Fujita, A. (1920). *J. Pharm. Soc. Japan*, **455**, 1.

Busing, W. R., Martin, K. O. & Levy, H. A. (1962). ORFLS, Oak Ridge National Laboratory, Oak Ridge, Tennessee, U.S.A.

Dunitz, J. D. (1949). *Acta Cryst.* **2**, 1.

Dunitz, J. D. & Schomaker, V. (1952). *J. Chem. Phys.* **20**, 703.

Fridrichsons, J. & Mathieson, A. McL. (1962). *Acta Cryst.* **15**, 119.

Harris, J. E. (1959). Dissertation Abstr. **20**, 887

Heyer, M. (1792). *Chemisch Journ. V. Crell*, **2**, 102; see also Wurtz, A. D. (1870). *Dictionnaire de Chimie*, **1**, 299. Paris: Librairie Hachette et Cie.

Hill, R. & Van Heyningen, R. (1951). *Biochem. J.* **49**, 332.

Karle, I. L. & Dragonette, K. S. (1965). *Acta Cryst.* **19**, 500.

Karle, I. L., Hauptman, H., Karle, J. & Wing, A. B. (1958). *Acta Cryst.* **11**, 257.

Karle, I. L. & Karle, J. (1963). *Acta Cryst.* **16**, 969.

Karle, I. L. & Karle, J. (1964). *Acta Cryst.* **17**, 1356.

Karle, I. L. & Karle, J. (1965). *Acta Cryst.* **18**, 345.

Karle, J. (1964). *In Advances in Structure Research by Diffraction Methods*, Vol. I. R. Brill, ed. New York: Interscience Publishers.

Karle, J. & Hauptman, H. (1953). *Acta Cryst.* **6**, 473.

Kartha, G. & Haas, D. J. (1964). *J. Amer. Chem. Soc.* **86**, 3630.

Lemaire, H. P. & Livingston, R. L. (1952), *J. Amer. Chem. Soc.* **74**, 5732.

Lustig, E. & Moriarty, R. M. (1965). *J. Amer. Soc.* **87**, 3252.

Margulis, T. N. (1965). *Acta Cryst.* **18**, 742.

Mathieson, A. McL. & Taylor, J. C. (1963). *Acta Cryst.* **16**, 524.

Moriarty, R. M., Romain, C. R., Karle, I. L. & Karle, J. (1965). *J. Amer. Chem. Soc.* **87**, 3251.

Owen, T. B. & Hoard, J. L. (1951). *Acta Cryst.* **4**, 172.