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The single isomorphous-replacement method: a correction.

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We wish to correct a fallacy contained in section 5 of our paper (Blow & Rossmann, 1961). It is stated there that it will be possible to decide the absolute configuration of the heavy atoms, so as to utilise the anomalous scattering effect, by correlation of the two Fourier syntheses, one based on single isomorphous replacement only, the other on anomalous scattering effects only. It is shown below that the correlation between these syntheses is exactly zero.

Let the electron-density distribution due to the single isomorphous replacement alone be given by

$$\varrho_{is}(\mathbf{x}) = \sum_h |\xi_h| \exp i(\alpha_h + 2\pi \mathbf{h}.\mathbf{x});$$

and the electron density distribution due to the anomalous scattering effects alone be given by

$$\varrho_{AD}(\mathbf{x}) = \sum_p |\eta_p| \exp i(\beta_p + 2\pi \mathbf{p}.\mathbf{x}) .$$

(Notation as in our paper.) The correlation between ϱ_{is} and ϱ_{AD} over the whole unit cell may now be defined as

$$C = \int_{\text{unit cell}} \varrho_{is}(\mathbf{x})\varrho_{AD}(\mathbf{x}) d\mathbf{x} .$$

Substituting the above Fourier expressions for ϱ_{is} and ϱ_{AD} we obtain

$$C = \sum_h \sum_p |\xi_h||\eta_p| \exp i(\alpha_h + \beta_p) \int_{\text{unit cell}} \exp 2\pi i(\mathbf{h}+\mathbf{p})\mathbf{x}.d\mathbf{x} .$$

The integral is zero unless $h+p=0$, when it becomes unity. Hence, making use of the Friedel relationship,

$$C = 2 \sum_h |\xi_h||\eta_h| \cos(\alpha_h - \beta_h) .$$

Fig. 4 of Blow & Rossmann (1961) shows that

$$\alpha_h - \beta_h = \pm \tfrac{1}{2}\pi. \text{ Hence } C = 0 \text{ always.}$$

The Fourier synthesis calculated with the correct configuration of heavy atoms will, of course, be more accurate than the one with incorrect absolute configuration. If only a single isomorphous pair is available, then the only criterion for determining the absolute configuration of the heavy atoms is the actual examination of the two possible Fourier syntheses for reasonable features.

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Variable weighting and the solution of the false structure of rubrofusarin.

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The molecular structure of rubrofusarin, a natural product, has been determined (Stout & Jensen, 1962) by a modification of Sayer's method (Sayer, 1952; Zachariasen, 1952). The unit cell parameters are

$$a = 7{\cdot}52,\ b = 23{\cdot}14,\ c = 7{\cdot}20 \text{ Å};\ \beta = 98{\cdot}2°.$$

The space group is $P2_1/c$, four molecules per unit cell. Fig. 1 shows the skeleton formula and numbering of the atoms.

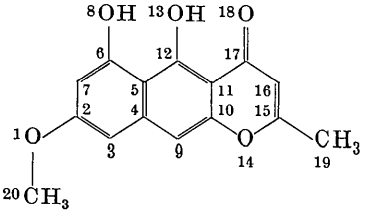

Fig. 1. Skeleton formula and numbering of the atoms.

In determining signs for 123 of the largest structure factors, it was found that they fell into two groups. For one, signs could be determined absolutely; for the other, they could be determined relative to a parameter $a = \pm$. Calculation of a three-dimensional F_0 synthesis with a taken $(-)$ led to a structure that refined by one F_o synthesis from $R = 46\%$ to $R = 30\%$ for 358 of the most intense reflections. At this point the structure appeared to have 'locked in', but several cycles of ΔF syntheses including only these terms for which $F_c > F_o/2$ did not decrease R below 22%. A determined effort to refine further using 1835 *hkl* data and again including only terms for which $F_c > F_o/2$ was without success. The molecular structure was reasonable, but inspection of the calculated structure factors revealed a few outstanding discrepancies that left no doubt the structure was incorrect.

Calculation of a three-dimensional F_o synthesis with a taken $(+)$ gave what appeared on first sight to be the same structure. Closer inspection proved, however, that it differed essentially in the displacement of the molecule with an average deviation between corresponding atoms in the two structures less than 0·25 Å. This structure refined normally by ΔF syntheses and a final full matrix least squares cycle (Busing & Levy, 1959).

Since the shortest Fourier component for complete Cu $K\alpha$ data has a period of 0·8 Å, one might reasonably question the reality of a false minimum corresponding to a structure with the average deviation of the atoms less than 0·25 Å from their true positions. The validity of such a minimum has been tested further using the full matrix least squares program of Busing & Levy on 1835 *hkl* reflections. Table 1 summarizes the results. In column 2 are listed for each atom in the incorrect structure the deviations from its position in the true structure

Table 1. *Summary of attempts to refine the false structure of rubrofusarin*

See text for explanation

1	2	3	4	5	6	7	8	9
Atom number								
1	0·099 Å	0·094 Å	0·101 Å	0·141 Å	0·071 Å	0·055 Å	0·029 Å	0·034 Å
2	0·135	0·159	0·149	0·137	0·110	0·052	0·068	0·012
3	0·276	0·293	0·296	0·284	0·259	0·212	0·125	0·057
4	0·228	0·244	0·181	0·196	0·235	0·151	0·122	0·057
5	0·360	0·354	0·340	0·346	0·163	0·137	0·076	0·043
6	0·239	0·266	0·215	0·194	0·255	0·130	0·027	0·054
7	0·296	0·245	0·238	0·189	0·131	0·079	0·041	0·034
8	0·272	0·184	0·204	0·180	0·162	0·098	0·077	0·038
9	0·278	0·323	0·314	0·287	0·263	0·192	0·124	0·069
10	0·264	0·251	0·277	0·179	0·314	0·179	0·090	0·055
11	0·316	0·356	0·346	0·362	0·205	0·192	0·100	0·044
12	0·244	0·284	0·240	0·208	0·167	0·089	0·037	0·013
13	0·199	0·206	0·201	0·164	0·082	0·052	0·020	0·017
14	0·382	0·247	0·249	0·207	0·285	0·181	0·070	0·035
15	0·204	0·131	0·129	0·134	0·063	0·100	0·068	0·061
16	0·236	0·241	0·225	0·212	0·189	0·121	0·071	0·037
17	0·253	0·212	0·211	0·186	0·170	0·125	0·078	0·029
18	0·151	0·152	0·150	0·128	0·120	0·084	0·031	0·025
19	0·131	0·184	0·163	0·168	0·111	0·084	0·055	0·036
20	0·150	0·082	0·090	0·126	0·134	0·074	0·072	0·050
Mean deviation	0·236	0·225	0·216	0·202	0·174	0·119	0·069	0·040
R	34·4%	31·1%	33·0%	33·0%	35·4%	30·3%	25·5%	22·4%

followed by the mean deviation and R. Succeeding columns give the same information for the following four tests, the false structure being the starting point for each.

1. In column 3 are summarized the results of 5 cycles with individual atom isotropic temperature factors. Comparison of the last two refinement cycles left no doubt that for all practical purposes refinement had ceased.

2. Similar to (1) except that an over-all isotropic temperature factor was used and 7 refinement cycles were carried through. Results are tabulated in column 4.

3. To the position of each atom in the false structure were applied *random* shifts approximately equal to the mean deviation from the true structure. The results after four refinement cycles are summarized in column 5. It is apparent that most of the atoms have refined essentially to their positions in the false structure.

In each of the above tests the weighting used was a modification of that conventionally used. All reflections with F less than about 25 e. were given unit weight, above that they were weighted by $(1/(0{\cdot}04)F)^2$.

4. A special patch was written to weight each reflection by $(|F_c|/|F_o|)^2$. Not only is any reflection for which $F_c > F_o$ weighted greater than 1, but unobserved reflections with $F_c > F_{\min}$ are weighted excessively. Unobserved reflections with $F_c < F_{\min}$ are given zero weight.

After the first refinement cycle using the variable weighting scheme, R had increased although the deviations in the atomic positions for most atoms had decreased appreciably, column 6. Successive cycles, columns 7 through 9, resulted in further improvement and after the fourth cycle R stood at 22·4%. Additional cycles resulted in slight oscillation but no further refinement. This is to be compared with the final refinement using conventional weighting for which $R = 18{\cdot}5\%$ for 1835 reflections as calculated by Busing & Levy's program, equivalent to $R = 15{\cdot}0\%$ if unobserved reflections are taken equal to $F_{\min}/2$.

The function minimized in all the least squares tests was $\Sigma w(\Delta F)^2$ where the summation is over the unrejected observations of non-zero weight.

These results confirm the earlier work with ΔF syntheses and show that a practical false minimum exists surprisingly near the true minimum in rubrofusarin. The difficulty was encountered in the present instance by the statistical method used in solving the structure. A minor group of the more intense reflections was only weakly related to the main group, and the reflections were assigned incorrect phases. While the particular behavior of rubrofusarin probably stems from the presence of a fairly definite subcell, many multiple ring and long-chain compounds may be expected to behave in a similar way.

It should be noted that the false structure of rubrofusarin cited here is not, in the usual sense of the terms, homometric or near homometric with the true structure although it behaved in a very similar way. For crystals with pronounced subcells, near homometric structures may be prominent, but they differ very appreciably from the true structure, e.g. by the displacement of the molecule by a bond length or more from its true position. The variable weighting function used to bring convergence for rubrofusarin could not be expected to work for such cases since the phases would not be sufficiently biased toward their true values. But other weighting functions, in particular those that weight low order reflections strongly, may be useful.

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