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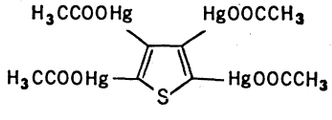
9 May 1972; revised 6 November 1972 ■

Image of a Sulfur Atom

Abstract. *Two-dimensional signal averaging has been applied to dark field electron micrographs of molecules of 2,3,4,5-tetraacetoxymercurithiophene. Only the mercury atom images are seen in single micrographs. However, in the composite image, resulting from photographic superposition of 64 individual images, the sulfur atom in the molecule is clearly revealed.*

Images of single atoms have now been obtained by four different techniques: field ion microscopy (*1*), scanning electron microscopy (*2*), and bright and dark field transmission electron microscopy (*3, 4*). All of the techniques image heavy atoms such as uranium (atomic number $Z = 92$), thorium ($Z = 90$), mercury ($Z = 80$), or tungsten ($Z = 74$). Lighter atoms such as iodine ($Z = 53$) or palladium ($Z = 46$) have been imaged with certainty only by the dark field technique (*4*). Even lighter substructures of molecules, substructures in which the heaviest atom was arsenic ($Z = 33$), have been revealed by signal averaging of their dark field images (*5*). Our results show that image processing of dark field electron micrographs of the molecule 2,3,4,5-tetraacetoxymercurithiophene can reveal an atom at least as light as sulfur ($Z = 16$).

The thiophene compound [made according to Palmer (*6*)] is planar in



structure. A chemical analysis of the molecule (*6*) yielded a ratio of mercury to sulfur atoms of 3.94 ± 0.09, very close to the expected ratio of 4. The location of the four mercury atoms in the structure of the molecule uniquely defines the position of the sulfur atom in the thiophene ring, even if the signal of the sulfur in the images of the molecule is completely masked by background noise. From model building with a Hg-C bond length of 2.07 Å and the experimentally determined structure of thiophene (*7*), the Hg-S distance should be 4.6 Å from the upper pair of mercury atoms (attached to C_2 and C_3) and 3.4 Å from the other two mercury atoms. Since the distance from the sulfur atom to each of the four mercury atoms is fixed, the restriction on the possible placement or misplacement of the sulfur atom in the superposition of any two images is very severe, making the molecule ideally suited for such a signal averaging process.

Dark field electron micrographs of the molecule were obtained on a Philips EM300 electron microscope at 80 kv and a magnification of 102,000. The objective aperture corresponded to a semiangular opening of 8.6×10^{-3} radian. Dark field conditions were obtained by tilting the incident beam about 0.75 degrees. Further details of the technique are given elsewhere (*4*).

Individual micrographs of the molecule are shown in Fig. 1, a to h. These images were chosen for the characteristic configuration and spacing of the four dark dots corresponding to the mercury atoms, as well as for the relative isolation of the group of dots from any neighboring configurations. Slight differences in configuration from one image to the next are easily accounted for by movement of the mercury atoms of as little as half an atomic diameter under the electron beam. Nevertheless, the constraint on the possible position of the sulfur atom is not lifted significantly. About 80 percent of the images showed configurations in which the distortion due to misplacement or movement of mercury atoms was more severe or in which the structure could be interpreted as a result of a tilted position of the planar molecule on the carbon support. Such images were not considered in the analysis, since the encroachment into the expected region of the sulfur atom of the rather strong image of a misplaced mercury atom could have biased the result.

Photographic superposition of four individual images (Fig. 1i) resulted in a marked relative decrease in the intensity of the background mottle around the molecule (*8*). Although no definite structure has emerged in the middle of the cluster of mercury atom dots, there is already some retention of signal in this region relative to the general background. This effect is enhanced when 16 individual images are superposed (Fig. 1j). Moreover, the reflection symmetry expected from the molecule is becoming more apparent in the image the greater the number of superpositions.

Since in the individual single images any observed asymmetry is thought to be due to displacement of atoms under the electron beam, rather than real structural asymmetry, the reflection symmetry of the molecule was used to increase the effective number of the image superposition with relatively little effort. The result is shown in Fig. 1k for 64 superpositions (32 individual images plus a reflection). In this image the background mottle is virtually absent while a definite signal has emerged at the position of the sulfur atom in the molecule.

In retrospect, the signal was already evident at 16 superpositions (Fig. 1j) and at 32 superpositions (not shown) without reflection. It is therefore not the result of a spurious effect of the symmetry operation. The reflection symmetry does appear to reinforce the fine detail suggesting further details in the image of the molecule. Such detail is, however, below the stated theoretical limit of resolution of the lens of the electron microscope and so should not be interpreted as representing structures of the molecule. It will be eliminated later (Fig. 1m).

Since the best superposition of the images had been chosen by visually aligning the marker mercury atom images, a check on this process was carried out by computer. The four images at the stage of 16 superpositions were digitalized in an array of 32 by 32 square elements, each element equivalent to an area of 0.25 Å^2. The photographic optical density range in each

image was divided into eight grades of darkening. If the optical density for corresponding elements of the four negatives was a_1, a_2, a_3, and a_4 the overlap was defined as

$$\sum_{i=1}^{4} \sum_{j=i+1}^{4} a_i a_j = a_1a_2 + a_1a_3 + a_1a_4 + a_2a_3 + a_2a_4 + a_3a_4$$

summed over the central array of 22 by 22 elements covering the molecular image plus a portion of the surrounding background. The overlap was calculated for lateral shifts of the images in two dimensions. Rotational movements of the images were considered, but the analysis, requiring longer computer times or more sophisticated and expensive machinery, was well beyond our budgetary means.

The image for the position of maximum overlap is shown in Fig. 1l. It differs from the image arrived at visually by only a single shift of 0.5 Å for each of two of the four images. The fine image detail is different from that in Fig. 1k, but again the density at the position of the sulfur atom is very pronounced, suggesting that at least the sulfur atom image has been revealed.

Lastly, since conservative estimates of the theoretical limit of resolution of the electron microscope used suggest that structures finer than 2.5 Å cannot be resolved (*9*), it appears reasonable that detail finer than this in the final image should be removed from the image by high-frequency filtration. This filtration was carried out by optical diffraction and reconstruction (*10*) by using a helium-neon laser (10 mw; Spectra Physics). Figure 1m shows the result of filtering the image in Fig. 1l with a resolution limit equivalent to 2.6 Å. Most of the fine detail has vanished. The strong images of the mercury atoms remain and so does that of the sulfur atom.

A comparison of the interatomic distances in Fig. 1m with those in the model mentioned above suggests that our image is 7 to 10 percent larger than expected. This result could have been anticipated, since in the selection of images configurations were discarded in which the mercury atom images encroached on the space of the sulfur atom too closely. Thus, molecules in which bombardment by the electron beam had tended to move the mercury atoms inward would not appear in the signal averaging process. Nevertheless, the agreement between the image and the model is excellent. A more exact comparison must await the x-ray crystallographic determination of the structure.

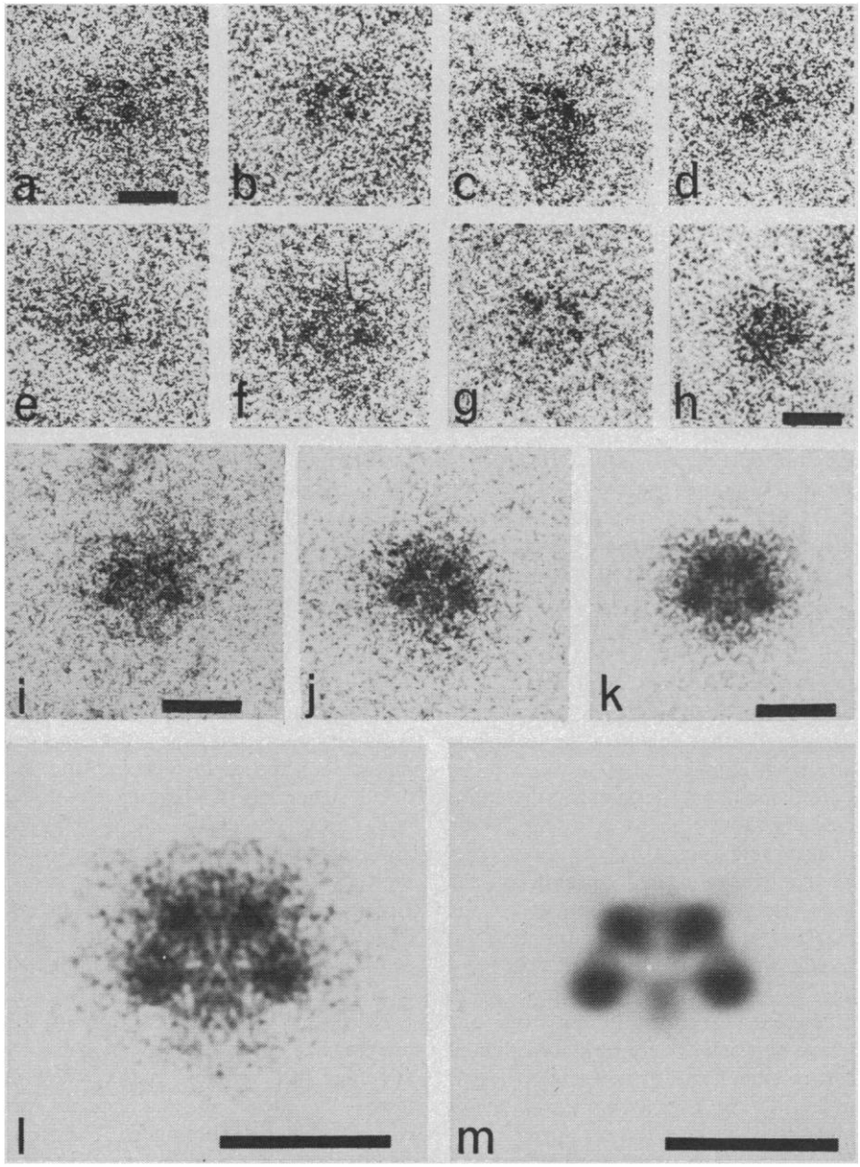


Fig. 1. (a to h) Dark field electron micrographs of eight individual molecules of 2,3,4,5-tetraacetoxymercurithiophene. (i to k) Visually aligned photographic superposition of (i) 4 molecular images, (j) 16 images, and (k) 64 images (32 individual molecules plus a reflection). (l) Computer assisted superposition of 64 images. (m) Image in (l) optically filtered to a resolution corresponding to 2.6 Å. (a to m) The scale is 10 Å.

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17 April 1972; revised 30 October 1972