Conformation of Aromatic Disulphides: the Molecular Structure of Ellman's Reagent

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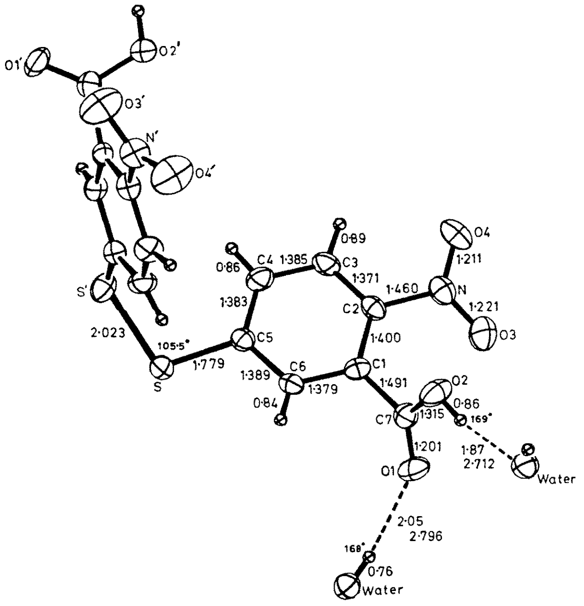
Summary The conformation of an aromatic disulphide linkage is best described by two torsion angles: the one involving the C–S–S–C atoms (usually near 90°) and that relating the orientation of the S–S bond to the plane of the phenyl ring (either *ca.* 0° or *ca.* 90°).

CERTAIN molecular features of disulphides in which the sulphur atoms are linked to unsaturated carbons, suggest that the interaction between the π-electron system and the disulphide bond may have an important effect on the molecular conformation of this linkage. The geometry of the disulphide bridge is of considerable biological significance, since the recently discovered sulphur-containing pyrimidine bases of tRNA[1] are susceptible to oxidation to their disulphides, which results in a change of the secondary structure and the activity of the nucleic acid.[2] In the present study the molecular structure of a typical aromatic disulphide, 5,5′-dithiobis-(2-nitrobenzoic acid), sometimes referred to as Ellman's Reagent, was determined by X-ray diffraction techniques. This compound is a widely used reagent for the determination of protein sulphydryl groups.[3]

Ellman's reagent crystallizes from aqueous ethanol as yellow orthorhombic prisms belonging to space group *P ccn*. The cell dimensions of these crystals are $a = 13{\cdot}816$ (0·001), $b = 11{\cdot}588$ (0·001), and $c = 10{\cdot}226$ (0·001) Å. The density measured by means of flotation (1·67 g./cm.^{3}) compares favourably with that calculated assuming four molecules of reagent plus four molecules of water in the unit cell (1·689 g./cm.³).

Intensity data were collected for 1555 independent reflections by the stationary crystal-stationary counter technique[4] using balanced filters for the Cu-K_α radiation (Ni *versus* Co). Of these reflections 1426 had intensities significantly greater than their respective backgrounds. The symbolic addition procedure was employed to derive the structure; use was made of a program written by Long.[5] The positional and thermal parameters of the various atoms including hydrogens were refined by least squares to an R value of 0·059 (for the observed data). The Figure gives a general view of the molecule showing intra- and inter-molecular bond lengths.



FIGURE

The molecular characteristics of the disulphide linkage in this molecule are compared with those of some analogous disulphides in the Table. The C–S–S–C torsion angle, ϕ_{SS} (defined in the manner of Klyne and Prelog[6]), is in the range of values observed for most open-chained disulphides, *i.e.* between 70° and 110°.[7] It is most probable that distortion of this dihedral angle away from 90° (minimum energy associated with this configuration accounted for by repulsion of lone-pair electrons on sulphurs) results from intermolecular forces of the nonbonded and bonded types within the solid. The 24° distortion most probably encompasses only a small energy requirement. In 5-UdRS$_2$ (for abbreviations see footnote †) the electronic nature of the S–S linkage appears to be a determinant of the magnitude of its ϕ_{SS} value, rather than intermolecular bonding.[8] The intramolecular bonding parameters about the S–S bond of Ellman's Reagent are in substantially good agreement with those found in 4-URS$_2$.

Comparative structural parameters for some disulphides

Compound†	ϕ_{SS}	S–S	C–S	C–S–S	$\phi_{\pi\text{-}SS}$	Ref
Ellman's Reagent	76·4°	2·023(2) Å	1·779(5) Å	105·5(2)°	16°	
4-URS$_2$	−87	2·022(4)	1·79(1)	104·0(4)	4(±3)[a]	9
5-UdRS$_2$	−50	2·108(3)	1·76(1)	102·0(2)	82(±6)[a]	8
APS$_2$	83	2·1	1·8	106	86[a]	11
P-C-SS-BA	81	2·046(3)	1·800(5)	105	20	13

[a] Average value.

In order to consider the orientation of the disulphide bond relative to the ring system to which it is attached, the conformation angle $\phi_{\pi\text{-}SS}$ is herein defined as the magnitude of the acute angle formed by the trace of the ring system projected down the C–S bond, with the trace of the S–S bond. In the four structures listed in the Table, the $\phi_{\pi-SS}$ value tends to fall into two ranges; those where the S–S linkage is almost coplanar with the ring (*ca.* 0°, *equatorial*) and those where it is approximately perpendicular to this residue (*ca.* 90°, *axial*). The existence of two distinct minima for the conformation about the C–S bonds in these disulphides raises an interesting possibility concerning the configuration of the intramolecular disulphide bridge formed in a tRNA^{tyr} isolated from *E. coli*.[2] It seems to us that in the disulphide of 4-thiouridine the more stable conformation about the C–S bond is equatorial as found in the crystal,[9] and this should also prevail in the case of the adjacent 4-thiouridines residues in a hexanucleotide fragment from tRNA^{tyr} where intermolecular disulphide formation is preferred over the intramolecular bonding.[10] The neighbouring 4-thiouridine moieties in the tRNA^{tyr} from *E. coli*[10] would require the *axial* conformation for $\phi_{\pi\text{-}SS}$ in the oxidized state of this nucleic acid, as is evident from model building. Therefore the secondary structure of the tRNA^{tyr} most probably permits the formation of the intramolecular disulphide bond to exist in what is possibly a slightly less favourable energetic state.

Though the intramolecular bond lengths and angles of the *o*-nitrobenzoic acid portion of the molecule are quite similar to those observed for *o*-nitrobenzoic acid itself,[12] there are striking differences in the conformations of the exocyclic functional groups. In both structures neither the nitro- or carboxyl moieties are coplanar with the phenyl ring. These groups are rotated from the plane of the benzene ring by 23° and 55° for the carboxyl and nitro-groups, respectively, in *o*-nitrobenzoic acid. The distortion in Ellman's Reagent is 70° for the carboxyl group and 20° for the nitro-residue. The conjugation of the *p*-disposed nitro- and disulphide groupings of atoms on the phenyl ring, may cause the overcrowding of the carboxyl and nitro-groups to be relieved in the opposite way to that in *o*-nitrobenzoic acid. The repulsion between these two exocyclic groups also causes the angles about the carbons to which they are attached to be distorted from trigonal symmetry. It is also interesting to note that the carboxyl group does not form hydrogen-bonded dimers as is found in crystals of

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† Abbreviations used: 4-URS$_2$, 4,4′-di(thiouridine); 5-UdRS$_2$, di-5-[1-(2′-deoxy-α-D-ribofuranosyl)uracilyl] disulphide; APS$_2$, di-(2-aminophenyl) disulphide; P-C-SS-BA, 2-)2-pyridylmethyldithiobenzoic acid.

o-nitrobenzoic acid. The water molecules along the two-fold axes form hydrogen bridges between one reagent molecule and another.

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