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THE FIVE-MEMBERED DISULPHIDE RING SYSTEM—I

GENERAL CHEMISTRY AND THERAPEUTIC ASPECTS

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Abstract—The specific properties of the 1:2-dithiolane ring system has been considered from the physicochemical point of view. The reactions of the five-membered cyclic disulphides have been correlated to those of other disulphides and special attention paid to the presumed relation to reactions of fundamental biochemical and physiological nature. Polarographic methods have been used to present evidence for the redox and ring stability properties of the five-membered ring system. A direct polarographic method of determining 1:2-dithiolane derivatives has been devised for biological systems.

CONSIDERABLE interest has in general been devoted to the study of organic disulphides, which seems well grounded from the theoretic organic-chemical point of view and the applied biochemical as well. The 1:2-dithiolane ring—the five-membered disulphide ring system—became an important unit in modern sulphur chemistry when its presence in 6-thioctic acid (α-lipoic acid) (I) was discovered.* The hypothesis advanced by Calvin and co-workers[2] about the function of α-lipoic acid in the photosynthesis has caused many efforts to determine the strain in the 1:2-dithiolane ring.

For a complete understanding of many of the disulphide properties it is, however, essential to know the steric arrangement of the bonds around the S—S group. This is chiefly determined by the electronic interaction between the lone-pairs on the sulphur atoms. Using unhybridized lone-pair electrons Pauling predicted a skew conformation for the disulphide group with energy minimum for dihedral angle 90° (i.e. the angle between the planes CSS and SSC). Using the molecular-orbital method, Bergson[3] could not only account for the skew conformation in the ground state but also for the red-shift found in ultra-violet spectra with decreasing dihedral angle. This theoretical treatment has also been extended to polysulphides and various forms of elemental sulphur.[4] Pauling's assumption about unhybridized lone-pair electrons, which was also used in the above-mentioned molecular-orbital treatments, is of course a rather rough approximation since the *bond angle* at sulphur is always greater than 90°. Hybridized lone-pair electrons have now been used as a basis in

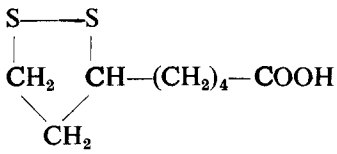
* For a review, see the article by Reed.[1]

molecular-orbital calculations on the sulphur–sulphur bond.[5] The same qualitative results are then obtained as regards the conformation and spectral changes although the torsion barrier in the S—S bond is smaller when sp^3-hybridized lone-pairs are used as compared with unhybridized orbitals. The hybridization is also proposed to be of importance for the reactivity of the S—S bond.

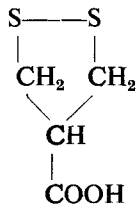
From the above considerations about the stabilized skew configuration of linear disulphides (for a representative figure, see Ref. 6) it can be readily understood that special properties must be attributed to five-membered cyclic disulphides. This is well illustrated by the work of Affleck and Dougherty[7] on the preparation and relative reactivites of the homologous unsubstituted saturated cyclic disulphides. Larger rings do not require any appreciable deviation from the normal value of the dihdral angle and therefore correspond more closely to the linear disulphides. This is further substantiated from the spectrochemical investigations of Calvin and co-workers[8] and one of the authors (L.S.) (see below). It is further strengthened by the calorimetric determinations of Sunner.[6]

In attempts to find methods to correlate significant properties of different types of disulphides to each other the spectrometric characteristics of related compounds have been examined. The investigations included determination of the ultra-violet and infra-red absorption spectra.[9] The special character of the 1:2-dithiolane ring system was clearly illustrated and the spectrometric differences could be interpreted with respect to the conformation data discussed above.

The results from the spectrochemical investigations and available details of a complete structure determination of 1:2-dithiolane-4-carboxylic acid (II) by Foss and Tjomsland[10] were used for a conformation analysis of the 1:2-dithiolane ring in



(I)



(II)

the acid (II) by Bergson and one of the authors (L.S.).[11] In this manner it was possible to present a critical discussion of the question about the steric strain in the five-membered disulphide ring system. Such a discussion is of fundamental importance for the further understanding and appraisal of Calvin's photosynthesis theory. It is also important from the biochemical and physiological point of view, as the tendency of the related compounds to undergo ring opening should be essential for the processes in question. The theoretic calculations have presented valuable indications here.

The ring stability and the redox properties of the S—S linkage disulphides are best studied with polarographic methods. It seemed likely that such an investigation would give accurate information on the real nature and special character of the 1:2-dithiolane system. A polarographic investigation was then started in order to get more information about the strength of the S—S linkage in the 1:2-dithiolane ring especially

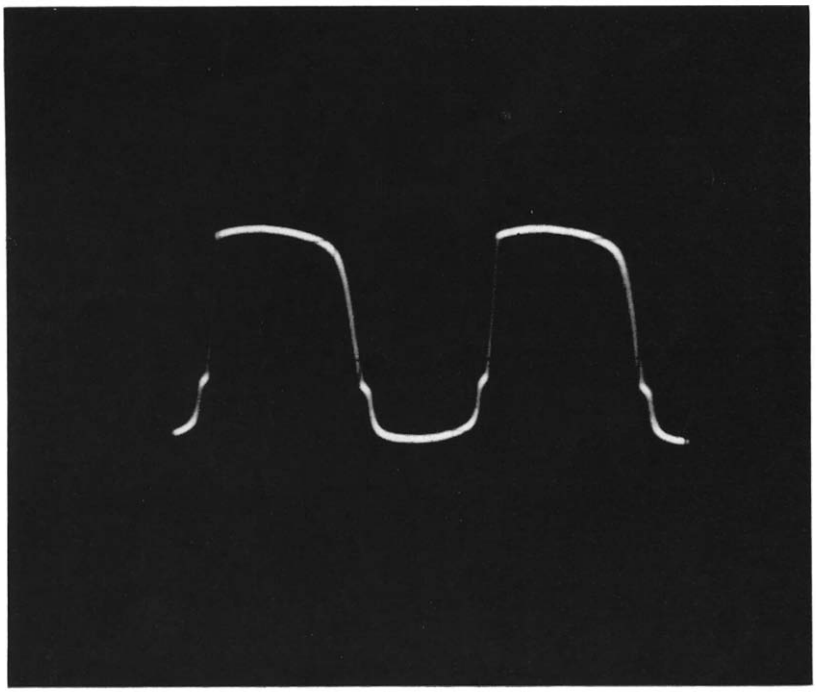


FIG 1. Oscillographic polarography. V–t-curve of $5{\cdot}0 \times 10^{-4}$ M dithio-pentaaerythrit in perchlorate solution. pH 1·2. Mercury-jet electrode.

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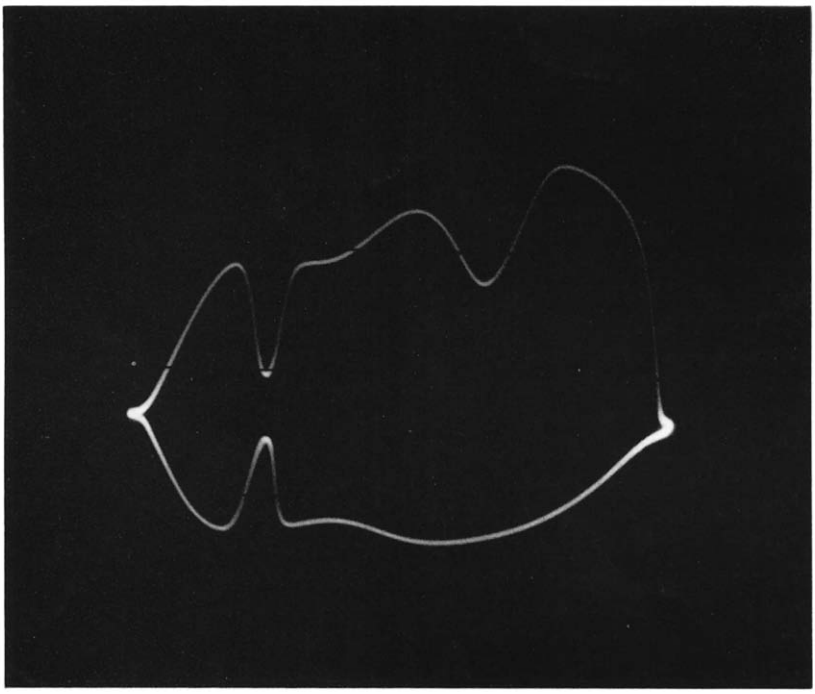
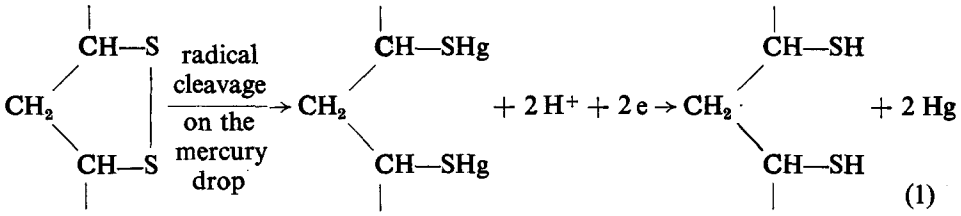


FIG. 2. Oscillographic polarography. dV/dt–V-curve of $5{\cdot}0 \times 10^{-4}$ M 4:4-bis-hydroxymethyl dithiolane in perchlorate solution. pH 1·2. Mercury-jet electrode.

compared with six- and seven-membered cyclic disulphides. Among several 1:2-dithiolane derivatives examined was also α-lipoic acid. Earlier we have established that five-membered cyclic disulphides are reversibly reduced at the dropping mercury electrode in a two-electron process.[9, 12] On the other hand the larger ring systems take part in an irreversible process at the same electrode. For the six- and seven-membered cyclic disulphides in comparison with the 1:2-dithiolane system a greater ring stability is apparent. A further comprehensive polarographic study of several 1:2-dithiolane derivatives has fully confirmed the unique electrochemical behaviour of this group in relation to other known disulphides. Polarographic investigations have been performed on aliphatic symmetric and unsymmetric disulphides and polysulphides. Cyclic polysulphides have also been included in this work. The influence of substitution (in 3-, 4- and 5-position) has been examined in detail on the electrode process for the five-membered cyclic disulphides. The results from all these investigations will be published in the near future.

The polarographic effects of the 1:2-dithiolane derivatives are all due to a reversible reduction of mercury compounds which are formed on the drops at the rupture of the S—S linkage. Schematically the electro-reduction proceeds according to:

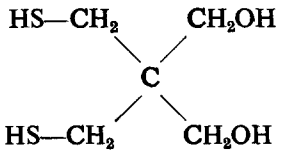


(1)

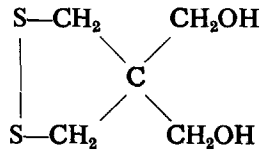
The radical (homolytic) cleavage of the S—S bond, must proceed almost instantaneously, if the mercury compound formed will be able to take part in a reversible process. This assumption has been confirmed in a satisfactory way with the aid of polarographic *i–t*-curves.

In the dithiolane ring the ideal reducing reaction of the S—S linkage may be more or less disturbed by substituents such as carboxyl groups in adjacent positions which can be shown from the shape of the corresponding polarograms. Without any substitution influence the value of $E_{\frac{1}{2}}$ (half-wave potential vs. SCE) for the cathodic wave is within the experimental limits equal to the same value for the anodic wave of the reduced form at the same pH. This fact indicates the reversibility of the electrode process.

Fig. 1 represents the *V–t*-curve of dithio-pentaaerythrit (III) and Fig. 2 the *dV/dt–V*-curve of 4:4-bis-hydroxymethyl-dithiolane (IV).



(III)



(IV)

These compounds represent the corresponding fully reduced and oxidized form and from the experiments it is quite clear that they take part in the same reversible reaction on the mercury electrode. From the oscillographic experiments it seems also reasonable to assume that the S—S bridge in the dithiolane ring is inclined to undergo more easily a radical cleavage than other types of disulphides. In this connection it is interesting to compare some other sulphur compounds in their polarographic behaviour. These are the sulphur systems thioacetic acid–dithio-diacetic acid,[13] cysteine–cystin[14–17] and glutathione[18, 19] in the reduced and oxidized state which are important from the biochemical point of view. In all these three systems the oxidized (disulphide) forms are irreversibly reduced to the corresponding thiols at the mercury drop according to the general formula

$$\mathrm{RSSR} + 2\,\mathrm{H^+} + 2\,e \xrightarrow{\text{irreversible}} 2\,\mathrm{RSH} \qquad (2)$$

On the other hand their reduced states are anodically and reversibly oxidized forming Hg(I)-compounds on the mercury electrode

$$\mathrm{RSH} + \mathrm{Hg} \underset{}{\overset{\text{reversible}}{\rightleftharpoons}} \mathrm{RSHg} + \mathrm{H^+} + e \qquad (3)$$

The pronounced irreversible character of the electrode reaction (2) indicates that in these cases a radical cleavage of the S—S bond does not proceed so readily as with the five-membered cyclic disulphides. In the open disulphides a greater strength of the S—S bond is apparent. This indication should be of physiological importance.

Ke[20] and also Sponar and Jirsa[21] have studied α-lipoic acid with polarographic methods without going into the electrode reaction in detail.

An interesting analogy supporting our theory can be found in the polarographic behaviour of certain diselenides. Diseleno-diacetic acid has been studied in detail by one of us (B.N.).[22] It has been found that a radical cleavage occurs with formation of electro-reducible Hg(I)-compounds on the mercury drop for this acid. Further, both open and cyclic diselenides are reduced according to the same mechanism. Then the main difference must be in the bond strength between aliphatic S—S and Se—Se groups as shown from their different ability to form reducible mercury compounds on the electrode surface. Such a difference in reactivity with mercury has already been established by Fredga.[23] From our experiments it is evident that the 1:2-dithiolane derivatives resemble the diselenides in their polarographic behaviour at a mercury electrode more than disulphides in general. Then this general property may be considered as a qualitative indication of the resemblance in bond strength of S—S and Se—Se groups for the chemical compounds in question.

A few years ago Weisberger and Suhrland[24–26] described the effect of selenium cystine on Murphy Lymphosarcoma tumour cells in rats and on leukaemia. Decreased availability of SH groups has been correlated with inhibition of tumour growth. Substances have also been found to inhibit cell division if they can oxidize SH compounds forming S—S bridges or are able to combine with SH groups to inactivate them. According to Weisberger and Suhrland it is possible that selenium cystine may work as a blocking analogue of cystine *in vivo*. Therefore it may affect cell growth and division, especially of malignant cells.

This hypothesis seems likely in the light of our knowledge from polarographic work about the diselenium bond strength. It is also probable that selenium cystine

like other diselenium compounds is able to interfere with redox processes with sulphur systems in ways favourable from the clinical point of view.

In their clinical experiments on acute leukaemia, Weisberger and Suhrland obtained some most striking and consistent results with selenium cystine. On the other hand, although their experiments seemed in the beginning to be very promising, severe symptoms of nausea and vomiting prevented the determination of its value in the chemotherapy of leukaemia.

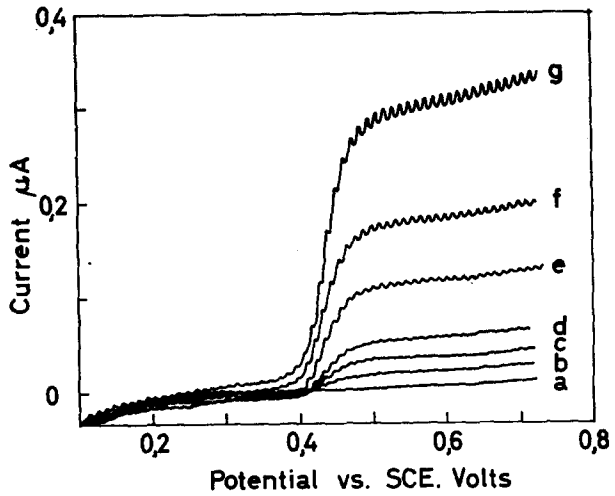


FIG. 3. 4;4′-Bis-hydroxymethyl-dithiolane of varying concentration in human plasma. Phosphate buffer, pH 7; (a) blank; (b) 2; (c) 4; (d) 6; (e) 12; (f) 18; (g) 30 μg per ml of plasma.

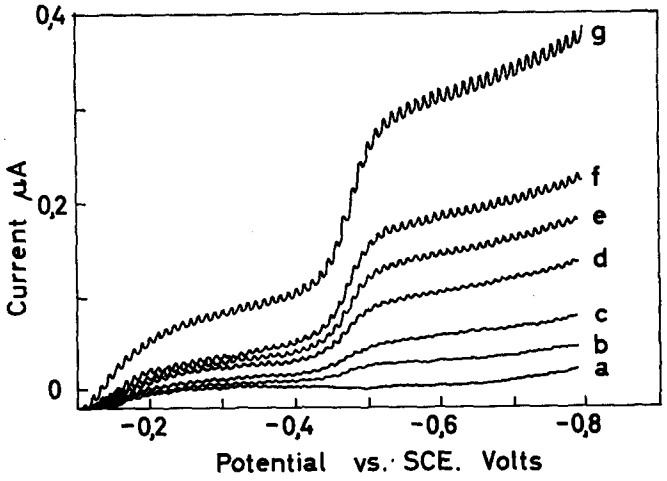


FIG. 4. 1:2-Dithiolanone-4-semicarbazone of varying concentration in human plasma. Phosphate buffer, pH 7; (a) blank; (b) 2; (c) 4; (d) 8; (e) 12; (f) 16; (g) 24 μg per ml of plasma. Calculated on the most negative wave.

If in the clinical experiments quoted above the main effect of the diselenium compounds is based on a redox process it might well be possible to get analogous results with less toxic disulphur compounds provided that they possess a S—S bond of such a strength that they can take part in a redox system in the same way as a Se—Se bridge. This idea led us to suggest pharmacological investigations of sulphur compounds with the 1:2-dithiolane ring system. During the last few years such experiments have been carried out by Kieler[27] in the Fibiger Laboratory and the results will be presented in separate papers in this journal. It should be mentioned that the general chemical behaviour and therapeutical experiences with α-lipoic acid are in agreement with our theory.

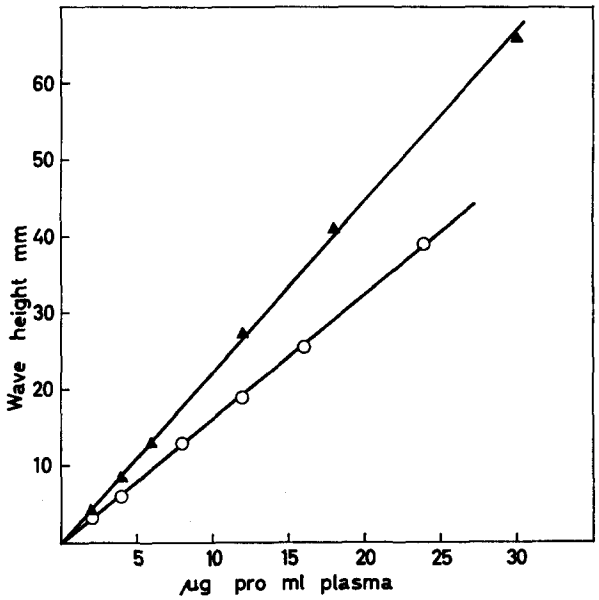


FIG. 5. Calibration curves of wave height in mm versus concentration in µg per ml in human plasma. (▲) 4:4′-Bis-hydroxymethyl-dithiolane. (○) 1:2-Dithiolanone-4-semicarbazone.

Finally it should be noted that polarography offers a simple and accurate analytical method for determining 1:2-dithiolane derivatives even in micro scale. The corresponding polarographic waves are in general well defined and the method can often be applied directly on complicated systems without any separation of the compound. Thus it has been possible to find a rapid method for determining 1:2-dithiolane derivatives in human plasma. This method is valuable in resorption studies in pharmacological and clinical tests. Figs. 3 and 4 represent the analytical application of the polarographic method on two dithiolane compounds for the determination of small quantities in human plasma. One is 4:4-bis-hydroxymethyl-1:2-dithiolane (Fig. 3) and the other 1:2-dithiolanone-4-semicarbazone (Fig. 4). From Fig. 5 it is evident that satisfactory linear relations exist between diffusion current and concentration.

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REFERENCES

1. L. J. REED, *Advanc. Enzymol.* **18**, 319.
2. M. CALVIN and J. A. BARLTROP, *J. Amer. Chem. Soc.* **74**, 6153 (1952).
3. G. BERGSON, *Ark. Kem.* **12**, 233 (1958); **13**, 11 (1958).
4. G. BERGSON, *Ark. Kem.* **16**, 315 (1960).
5. G. BERGSON, *Ark. Kem.* (1961). In press (where a full list of references concerning the problem of internal rotation, ultra-violet spectra and reactivity is given).
6. S. SUNNER, *Svensk Kem. Tidskr.* **67**, 513 (1955).
7. J. G. AFFLECK and G. DOUGHERTY, *J. Org. Chem.* **15**, 865 (1950).
8. J. A. BARLTROP, P. M. HAYES and M. CALVIN, *J. Amer. Chem. Soc.* **76**, 4348 (1954).
9. L. SCHOTTE, *Ark. Kem.* **9**, 441 (1956).
10. O. FOSS and O. TJOMSLAND, *Acta Chem. Scand.* **11**, 1426 (1957); and private communications.
11. G. BERGSON and L. SCHOTTE, *Ark. Kem.* **13**, 43 (1958).
12. B. NYGÅRD and L. SCHOTTE, *Acta Chem. Scand.* **10**, 469 (1956).
13. D. L. LEUSSING and I. M. KOLTHOFF, *J. Electrochem. Soc.* **100**, 334 (1953).
14. I. M. KOLTHOFF and C. BARNUM, *J. Amer. Chem. Soc.* **62**, 3061 (1940).
15. I. M. KOLTHOFF and C. BARNUM, *J. Amer. Chem. Soc.* **63**, 520 (1941).
16. O. GRUBNER, *Coll. Czech. Chem. Communs.* **19**, 444 (1954).
17. M. KALOUSEK, O. GRUBNER and A. TOCKSTEIN, *Coll. Czech. Chem. Communs.* **19**, 1111 (1954).
18. W. STRICKS and I. M. KOLTHOFF, *J. Amer. Chem. Soc.* **74**, 4646 (1952).
19. I. TACHI and S. KOIDE, *Internat. Polarographic Congress Prague*, vol. I, 386 (1951).
20. B. KE, *Biochim. et Biophys. Acta* **25**, 650 (1957).
21. J. SPONAR and M. JIRSA, *Biochim. et Biophys. Acta* **29**, 434 (1958).
22. B. NYGÅRD, *Acta Chem. Scand.* (1961). In press.
23. A. FREDGA, *Ark. Kem.* B. **11**, No. 44 (1934).
24. A. WEISBERGER, L. SUHRLAND and J. SEIFTER, *Blood* **11**, 1 (1956).
25. A. WEISBERGER and L. SUHRLAND, *Blood* **11**, 11 (1956).
26. A. WEISBERGER and L. SUHRLAND, *Blood* **11**, 19 (1956).
27. J. KIELER, *Biochem. Pharmacol.* **11**, 453 (1962).