# Chiroptical Properties of 2,2'-Dithio- and 2,2'-Diseleno-bisacetic Acids *

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Circular dichroism (CD) studies of 2,2'-dithiobisacetic acids substituted with alkyl or phenyl groups and their diselenide analogues show that considerable interaction occurs between the disulfide (or diselenide) chromophore and the carboxyl or phenyl groups, giving rise to intense Cotton effects (CE's) which dominate the near UV region of the CD spectrum. In contrast, when the disulfide and carboxyl chromophores are separated by two carbon atoms, each chromophore gives a separate CE of normal intensity at the expected wavelength with no evidence of interaction between them.

The optical rotatory properties of the disulfide group have generated much recent attention[1] because of the importance of the group in the formation of bridges between polypeptide chains and its presence in many natural and synthetic compounds of biological interest.[2] Most of the optical activity measurements on chiral disulfides have dealt with the relationship between the sign of the Cotton effect (CE) and the angle of twist of the disulfide chromophore. In aliphatic strain-free disulfides, having dihedral angles of about 90°, the optical activity due to inherent disulfide chirality is small.[3] In this case perturbations due to chirally placed vicinal groups become important as a contributor to the observed optical activity.[3,4] The vicinal perturbations may be static as in the case of a
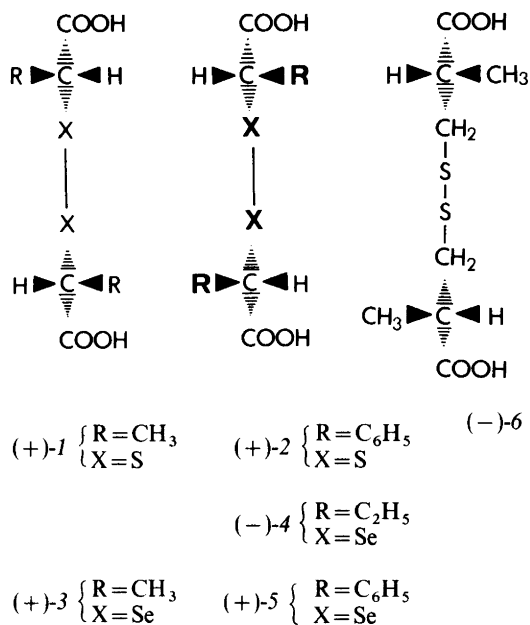
charged moiety, e.g. $^+NH_3$ of cystine at low pH, or they may be dynamic in nature such as may exist in the coupling of disulfide transitions with, e.g., tyrosine transitions in polypeptides.[5] Woody's treatment[6] suggests a high sensitivity of the disulfide transitions to coupling with transitions in other chromophoric groups in the chiral environment. Such couplings are especially important in complex disulfides e.g. proteins,[7] peptide hormones[5] and certain antibiotics,[8] but are perhaps more easily identified in small molecules.

Diselenides, while absorbing at longer wavelengths than the corresponding disulfides,[9-11] are closely analogous to disulfides in many respects and their ultraviolet absorption in particular is caused by similar electronic excitations. The theoretical considerations[3,6] on chiral disulfides may therefore be applied also to diselenides.[10,12]

Extensive studies of the electronic absorption properties of disulfides[11,13] and diselenides[9-11] have shown that the introduction of carboxyl groups at the α-positions of the dialkyl disulfides and diselenides is accompanied by considerable changes in the absorption spectra. Thus peaks appear which are not characteristic of either unsubstituted disulfides (diselenides) or simple carboxylic acids, despite the non-conjugative positions of the groups. A similar phenomenon was observed[14,15] for monosulfides with different groups e.g. carbonyl, carbalkoxy and phenyl and for some monoselenides.[16] Therefore it appears that considerable interaction can take place between sulfur and selenium and a number of suitably located chromophoric groups. In order to investigate how

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COOH      COOH      COOH

R ▶C◀ H    H ▶C◀ R    H ▶C◀ CH₃

X         X        CH₂

|          |        S

X         X        S

H ▶C◀ R    R ▶C◀ H    CH₂

COOH      COOH   CH₃ ▶C◀ H

                         COOH

$(+)\text{-}1$ $\begin{cases} R=CH_3 \\ X=S \end{cases}$   $(+)\text{-}2$ $\begin{cases} R=C_6H_5 \\ X=S \end{cases}$   $(-)\text{-}6$

                     $(-)\text{-}4$ $\begin{cases} R=C_2H_5 \\ X=Se \end{cases}$

$(+)\text{-}3$ $\begin{cases} R=CH_3 \\ X=Se \end{cases}$   $(+)\text{-}5$ $\begin{cases} R=C_6H_5 \\ X=Se \end{cases}$

such interactions may manifest themselves in the chiroptical properties of disulfides and diselenides, we have measured the circular dichroism (CD) spectra of some 2,2′-disubstituted 2,2′-dithiobisacetic acids (Table 1, *1* and *2*) and their diselenide analogues (*3* − *5*), which in addition to the disulfide or diselenide groups contain a carboxyl and in two cases also a phenyl chromophore. For purposes of comparison 2,2′-dimethyl-3,3′-dithiobispropionic acid (*6*), in which the carboxyl groups are at the β-positions to the disulfide group, was also studied.

## RESULTS AND DISCUSSION

The absolute configuration of (+)-2,2′-dithiobis-propionic acid [17] [(+)-*1*] is known to be R,R from reduction [18] of (+)-*1* to (+)-2-mercaptopropionic acid, which is configurationally identical with R-lactic acid.[19] Dextrorotatory 2,2′-diselenobispropionic acid [20] [(+)-*3*] has been shown [21] to have the same configuration as (+)-*1*. Although the configuration of (−)-2,2′-diselenobisbutyric acid [20] [(−)-*4*] has not previously been established, from the present CD measurements (Table 1) the configuration can be assigned as S,S. The similarity in the rotational shifts of 2,2′-dithiobisphenylacetic acid [22] (*2*) and 2,2′-diselenobisphenylacetic acid [20] (*5*) in different solvents at the sodium D-line was taken as evidence for the configurational identity of the acids with the same sign of rotation,[20] although the absolute configuration of the acids remained unknown. Optical rotatory dispersion (ORD) measurements [23] down to 300 nm support these conclusions. Oxidation of (−)-2-mercaptophenylacetic acid, whose absolute configuration has been rigorously established as R,[24] with hydrogen peroxide in acetic acid has now been shown to give (−)-*2*, which must therefore have the R,R configuration. On the basis of the present CD measurements of the acids (+)-*2* and (+)-*5*, the configuration of (+)-*5*, can now safely be assigned as S,S. The configuration of (−)-2,2′-dimethyl-3,3′-dithiobispropionic acid [(−)-*6*] is known to be S,S.[25]

The CD maxima of compounds *1* − *5* are shown in Table 1. All compounds give intense CE's in the 225 − 245 nm region, where neither the isolated S − S or Se − Se chromophore, nor the carboxyl

*Table 1.* Circular dichroism of 2,2′-dithio- and 2,2′-diselenobisacetic acids.

| Com-pound | X | R | Absolute configuration | H₂O(pH 0.5) | | H₂O(pH 4.0) | |
|---|---|---|---|---|---|---|---|
| | | | | $[\theta]^a$ ($\lambda_{max}$, nm) | R/10⁴⁰ c.g.s.[b] | $[\theta]^a$ ($\lambda_{max}$, nm) | R/10⁴⁰ c.g.s.[b] |
| (+)-*1* | S | CH₃ | (R,R) | +44310(225) | +58.4 | +36600(227) | +41.9 |
| (+)-*2*[c] | S | C₆H₅ | (S,S) | +156150(227)[d] | +153 | +149650(227)[d] | +149 |
| (+)-*3* | Se | CH₃ | (R,R) | +343(330)[e] | | +69(330)[e] | |
| | | | | +34790(239) | +57.8 | +19055(247) | +21.9 |
| | | | | | | −45000(195) | |
| (−)-*4* | Se | C₂H₅ | (S,S) | −420(341) | | −301(344) | |
| | | | | −42050(240) | −67.1 | −30100(245) | −40.0 |
| | | | | | | +24000(204)[f] | |
| (+)-*5*[c] | Se | C₆H₅ | (S,S) | +102420(242) | +131 | +103940(241) | +132 |

[a] Molar ellipticity. [b] Rotatory strength. [c] Measured in 50 % ethanol at the pH indicated. [d] This band has shoulders at 260, 267 and 273 nm. [e] Shoulder. [f] Shortest wavelength reached.

or phenyl chromophore have strong absorption maxima. In the disulfides this intense CE extends over the 250 nm region and conceals any optical activity associated with the long-wavelength disulfide absorption band, but in the diselenides (+)-*3* and (−)-*4* a CE corresponding to the lowest energy Se−Se absorption band is clearly visible.

In (+)-*1*, whose CD spectrum in methanol has previously been reported,[26] there is considerable evidence that the intense CE near 225 nm arises from an interaction between the carboxyl group and the disulfide chromophore. Thus the CE is absent in the spectrum of the corresponding alcohol.[26] For the acid an increase of pH from 0.5, where the carboxyl group is completely unionized, to 4 where the carboxyl group is mostly ionized (p$K_a$ for a carboxyl group $\alpha$ to a disulfide moiety was found to be about 3.4[27]) leads to a substantial decrease in intensity and a red shift of the CE (Table 1). Organic disulfides, especially those containing an acidic proton $\alpha$ to sulfur, are known to undergo alkaline decomposition.[28,29] However, the decrease in intensity of the 225 nm CE of (+)-*1* on raising the pH cannot be due to such decomposition since (+)-*2*, which is even more sensitive to alkali than (+)-*1*,[29] did not give a significant decrease in the intensity of its CE under similar conditions.

Further evidence that an interaction between the carboxyl group and the disulfide chromophore is responsible for the 225 nm CE of (+)-*1* comes from the CD spectrum of (−)-*6* (Fig. 1), in which a second carbon atom has been inserted between the disulfide chromophore and the carboxyl group. The CD-spectrum of (−)-*6* in water at pH 0.5 exhibits a negative CE at 259 nm ([$\theta$] = −1315), which is at slightly longer wavelength than the electronic absorption maximum ($\lambda_{max}$ 250 nm, $\varepsilon$ 400 in water at pH 0.5). The position, sign and magnitude of this CE agree with those of the long-wavelength CE of the closely related L-cystine.[30,31] On this basis[31] we may similarly assign the 259 nm CE of (−)-*6* to transitions of the disulfide chromophore. The position (210 nm) and magnitude ([$\theta$] = −4570) of the short-wavelength CE of (−)-*6* are typical for the $n \rightarrow \pi^*$ transition of a carboxyl group,[32] which suggests that sulfur−carboxyl interactions are virtually absent in (−)-*6*. L-Cystine shows a fairly intense CE at about 220 nm, ascribed to an $n \rightarrow \pi^*$ transition of the carboxyl group.[30,31] The red shift of this CE relative to its position in other amino acids was explained[31] by vicinal carboxyl−
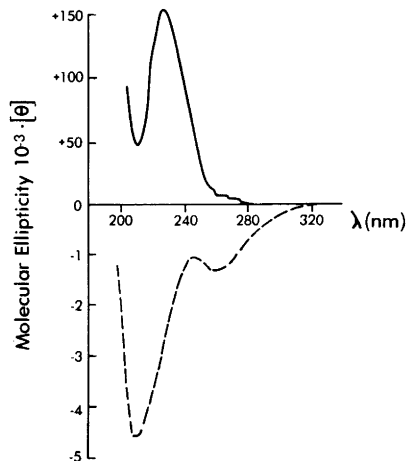
*Fig. 1.* CD spectra of (−)-2,2′-dimethyl-3,3′-dithio-bispropionic acid [(−)-*6*] in water (pH 0.5) (———) and (+)-2,2′-diphenyl-2,2′-dithiobisacetic acid [(+)-*2*] in 50 % ethanol (pH 0.5) (———).

sulfur group electronic interactions, but it appears, in view of the results obtained with (−)-*6*, that other factors may also contribute.

The very high intensity of the CE of (+)-*2* (Fig. 1) is that normally associated with an inherently dissymmetric chromophore. Moscowitz[33] has calculated that the rotational strength of such chromophores should be of the order of $10^{-38}$ c.g.s. Since both the magnitude and position of the CE are unaffected by an increase of pH (Table 1) it appears that the CE is generated primarily by an interaction between the disulfide chromophore and the phenyl group. Similar interactions have been observed in benzyl ethyl sulfide, whose electronic absorption spectrum exhibits a shoulder at about 235 nm, not present in the spectrum of phenethyl ethyl sulfide.[14]

In the diseleno acids (+)-*3* and (−)-*4* the intense CE is bathochromically shifted about 15 nm (pH 0.5) compared to its position in (+)-*1* and coincides with the electronic absorption maxima[11] of the compounds. The red shift and decrease in intensity of the CE on raising the pH are more pronounced than for the disulfide (+)-*1* (Fig. 2). These CE's most likely are responsible for the high D-line rotations of the compounds.[20] The large decrease in D-line rotations [(+)-*3* even gives an inversion of sign of rotation] observed[20] on going from dilute HCl to a fully neutralized water solution
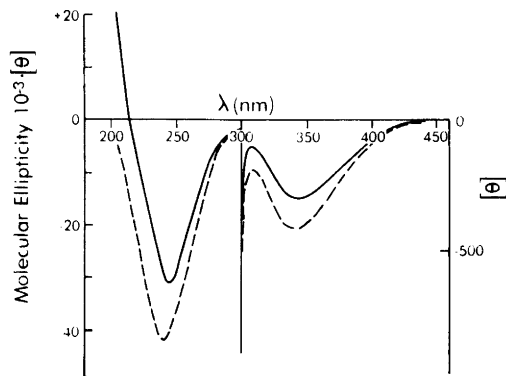
*Fig. 2.* CD spectra of ( − )-2,2'-diselenodibutyric acid [( − )-*4*] in water (— — —) pH 0.5; (————) pH 4.

further illustrates the sensitivity of the CE's to pH changes. A weak CE associated with transitions of the Se − Se chromophore[10,11] is observed at 330 − 340 nm, at longer wavelength than the shoulder in the electronic absorption spectra.[10,11] At pH 4, an additional strong CD band, observed at 195 nm, corresponds roughly in position with the highest energy CE observed for selenocystine at pH 5.5, and assigned to a transition within the Se − Se chromophore.[12]

Except for a red shift of *ca.* 15 nm and a slight decrease in rotational strength, the diselenide ( + )-*5* shows a CE similar to that of ( + )-*2*, again unaltered in position and intensity by an increase of pH.

Although no experimental evidence concerning the preferred conformations of the acids is available, the dihedral angles of the S − S and Se − Se groups can be assumed to be close to 90° (perhaps somewhat larger due to a slight interaction between the substituents[10]). At these dihedral angles, the contribution to the optical activity from inherent disulfide and diselenide chirality is small[3] as evidenced by the low optical activity of the long-wavelength disulfide and diselenide absorption bands. Instead strong CE's arising from dynamic coupling of disulfide and diselenide transitions with transitions within the carboxyl and phenyl groups dominate the CD spectra in the near-ultraviolet region for compounds *1* − *5* in which the interacting chromophores are attached to the same carbon atom. However, when the two chromophores are separated by two carbon atoms, as in *6*, each chromophore gives a separate CE of normal

intensity at the expected wavelength with no evidence of interaction between them. In compounds having both a carboxyl and a phenyl group attached to the chiral center, the phenyl moiety seems to give the strongest coupling interaction with the disulfide and diselenide groups, as shown by the high intensity of the CE in *2* and *5* and its lack of dependence on pH (Table 1).

## EXPERIMENTAL

CD measurements were made on a JASCO J-500A spectropolarimeter or a Roussel-Jouan Dichrograph II at 20 °C and at concentrations of 0.03 − 0.3 %. CD curves are the average of at least two separate measurements. Rotations were measured at 25 °C on a Perkin-Elmer 141 polarimeter using a 1 dm cell.

*(R,R)-( − )-2,2'-Diphenyl-2,2'-dithiobisacetic acid* [*( − )-2*]. A solution of *R*-( − )-α-mercaptophenylacetic acid[24] [0.0845 g, $[\alpha]_D^{25}$ − 106.2° (c. 0.5, 95 % ethanol), 80 % optically pure[24]] in 0.48 ml acetic acid was treated with hydrogen peroxide (0.027 ml, 30 %) in 0.36 ml acetic acid, and the mixture allowed to stand at room temperature for 2.5 h. Excess water was added and the solution extracted with ether. The extracts were washed ($H_2O$) and dried ($Na_2SO_4$) and the solvent removed leaving a solid (0.082 g) which crystallized from aqueous ethanol in white plates of the (R,R)-( − )-disulfide, m.p. 208 − 210 °C, $[\alpha]_D^{25}$ − 215.8° (c. 0.6, ethanol). Lit.[22] $[\alpha]_D^{25}$ − 253.8° (c. 1.1, ethanol). *Anal.* Found: S 19.05. Calc. for $C_{16}H_{14}O_4S_2$: S, 19.17 %. Our sample of ( + )-*2* had m.p. 213 − 214 °C, $[\alpha]_D^{25}$ + 261.3° (c. 0.6, ethanol).

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