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THE TEMPERATURE-DEPENDENT CIRCULAR DICHROISM SPECTRUM OF R-(+)-α-LIPOIC ACID. CONFORMATIONAL ANALYSIS OF THE DISULFIDE-CONTAINING FIVE-MEMBERED RING.[1]

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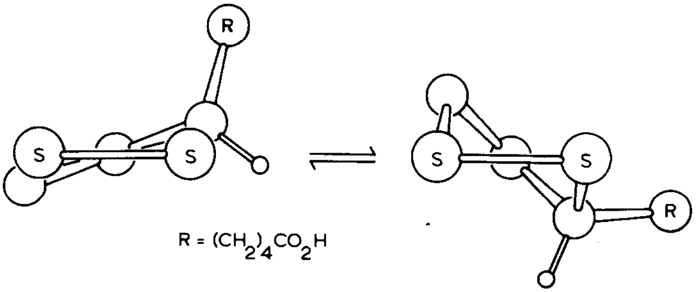
Circular dichroism (CD) investigations of disulfides with CSSC dihedral angle, ϕ, of 60, 30, and 0° demonstrate that as ϕ is reduced from 60 to 0° the longest wavelength CD band, band 1, shifts from ~290 to 370 nm and decreases in intensity.[2] When $\phi > 0°$ the sign of band 1 is determined by the chirality of the disulfide. The CD spectrum of M (left-handed) chiral disulfides with ϕ ~ 30° is characterized by negative bands at 330 and 215 nm and a positive band at 262 nm. The CD spectrum of R-(+)-α-lipoic acid (**I**)[3] is anomalous in comparison, exhibiting (in isooctane) very weak bands at 354, 310, 262, 239, and 216 nm. The CD spectra of **I** and methyl 2,3-O,O-diacetyl-4,6-dideoxy-4,6-epidithio-α-D-galactoside (**II**)[2], $\phi \approx 30°$, are compared in Figure 1. The two oppositely signed bands in the 280-400 nm region in place of a single more intense band can result from the overlap of two bands of greater intensity and opposite in sign separated by an interval that is small in comparison to the observed separation (Figure 1).[4]

To account for the CD spectrum of **I** we propose an equilibrium between two dominant conformations, **Ia** and **Ib**. In **Ia** the disulfide has P chirality with the side-chain R pseudo-axial to the ring. This conformation should have positive bands at ca 330 and 216 nm and a negative band at ca 260 nm. In **Ib** the disulfide has M chirality with the side-chain pseudo-equatorial.



R = $(CH_2)_4CO_2H$

Ia Ib

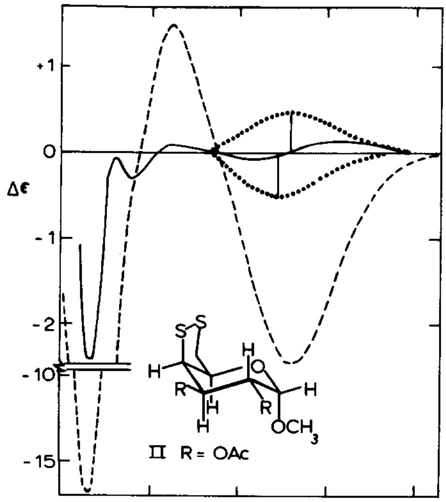


Figure 1. The CD spectrum of I in isooctane at 28°C (——) and the model compound, II, in hexane (---). The spectrum of I above 290 nm can be represented by the sum of two bands (···).

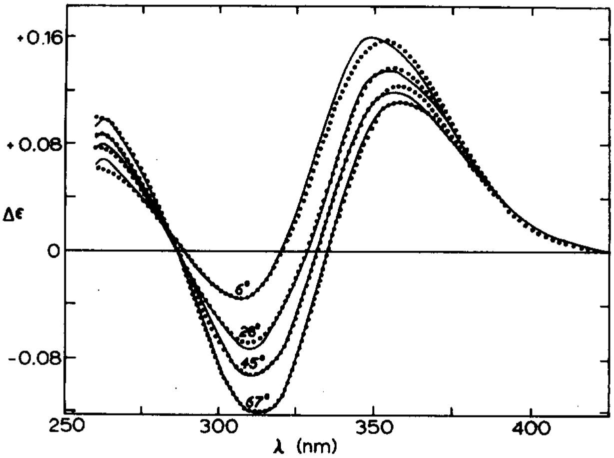


Figure 2. Observed (——) and calculated (···) temperature-dependent CD spectrum of I in isooctane. Temperature in °C. The equilibrium constants K (K = Xa/Xb) for the calculated spectra are 1.33 (67°), 1.43 (45°), 1.51 (28°), and 1.65 (6°).

The CD spectrum of Ib will be similar to that of Ia but opposite in sign. In addition, since the first band (354 nm) is positive, we can conclude that the dihedral angle is smaller in Ia than in Ib.

The CD spectra of I in isooctane at four different temperatures are shown in Figure 2. In going from 67 to 6°C the positive band at 358 nm increases in intensity and shifts to 349 nm while the negative band at 314 nm decreases in intensity and shifts to 308 nm, indicating that Ia, with the smaller dihedral angle and pseudo-axial side-chain, is thermodynamically more stable.

To test the reasonableness of these conclusions, the four spectra were fit simultaneously to a model which includes the CD spectral parameters for both Ia and Ib as well as the thermodynamic parameters for the equilibrium Ia $\rightleftarrows$ Ib. While primarily interested in spectral parameters for band 1 (~330 nm), we included parameters for band 2 (~260 nm) in the model to provide an appropriate temperature-dependent background in the 260-325 nm region. The bands were assumed to be Gaussian when $\Delta\varepsilon$ is plotted against λ. The CD spectrum of each conformation is assumed to be temperature-independent. The temperature-dependent spectra of the equilibrium mixture

	Ia	**Ib**	**II**(H)	**II**(M)	**II**(W)
$\lambda°$ (nm)	329	320	330	329	327
$\Delta°$ (nm)	40	34	34	35	34
$\Delta\varepsilon°$	+0.79	-1.24	-2.45	-1.53	-0.70
$R \times 10^{40}$	+3.8	-5.3	-10.0	-6.6	-3.0

Table 1. Calculated spectral parameters of the lowest energy transition for **Ia** and **Ib** compared with observed parameters for **II** in hexane (H), methanol (M), and water (W) solvent.

were obtained by combining the spectra of **Ia** and **Ib** in proportion to their respective mole fractions, X_a and X_b, which are calculated assuming a Boltzmann distribution where $X_a/X_b = K = \exp(-\Delta G/RT)$. Since ΔG appeared to be temperature-dependent, the enthalpy and entropy differences, ΔH and ΔS, were used as parameters where $\Delta G = \Delta H - T\Delta S$. Initial parameter values were varied using an iterative procedure[1] until a best fit of calculated and observed spectra was obtained (Figure 2). The estimated spectral parameters for the lowest energy transition of **Ia** and **Ib** are in the range of those observed for **II** in various solvents (Table 1). The X-ray structure of (±)-α-lipoic acid[5] shows that, in the crystal, molecules have conformation **Ib** with $\phi = 35°$ in excellent agreement with $\lambda°$ of 320 nm for this conformation. Since $d\phi/d\lambda° = -0.75$ deg/nm,[2] we estimate that ϕ for **Ia** is 6.4° smaller than **Ib** or ~29°, slightly larger than the 27° observed for crystalline 1,2-dithiolane-4-carboxylic acid.[6] The absolute value of $\Delta\varepsilon°$ or rotational strength $\underline{R}$ is larger for **Ib** than for **Ia**, consistent with the observation in model compounds that the intensity decreases as ϕ becomes smaller.[2] The enthalpy difference, ΔH, of 670 cal/mole favors **Ia** over **Ib**. The higher disulfide torsional energy for **Ia** is apparently more than balanced by minimization of other structure-energy parameters in the molecule. The entropy difference, ΔS, of 1.4 cal/deg mole stabilizes **Ib** over **Ia** and possibly results from loss of rotational freedom in the side chain when in the pseudo-axial conformation. The free energy difference ($-\Delta G$) at 25°C is 250 cal/mole, giving an equilibrium constant of 1.50 (or X_a/X_b = 60/40). By comparing the spectrum of **I** in dioxane with a series of spectra generated from the calculated spectral parameters for **Ia** and **Ib**, varying K from 1.0 to 1.5, we estimate that in dioxane $K_{26°} \approx 1.17$ ($-\Delta G$ = 95 cal/mole). This is consistent with the temperature-dependent spectrum in dioxane where the 364 nm band increases in intensity as the temperature is lowered, but to a much lesser extent than observed in isooctane. Spectral parameters of **I** in isooctane and dioxane are compared in Table 2.

It is interesting to note that although the pseudo-axial conformation is only slightly in excess in lipoic acid, it is the dominant conformation in both 1,2-dithiolane-3-carboxylic acid[7] and 1,2-dithiolane-3,5-dicarboxylic acid.[8] While the lowest energy CD band (330 nm) of these compounds appears normal when compared with spectra of model compounds, the higher energy bands (260 and 230 nm)[2] are strongly perturbed by the adjacent carboxyl group and are not representative of the "isolated" disulfide.

I in isooctane (28°C)	354(+0.136)	310(-0.072)	262(+0.080)
I in dioxane (26°C)	364(+0.082)	315(-0.16)	259(+0.10)

Table 2. Observed CD band parameters, λ°(Δε°), of **I**.

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REFERENCES:

(1) Taken in part from the Ph.D. Dissertation of L. A. Neubert, Indiana University, 1969. Diss. Abstr. Int. B, **30**(5), 2093 (1969).

(2) L. A. Neubert and M. Carmack, J. Amer. Chem. Soc., **96**, 943 (1974).

(3) The absolute configuration of (+)-lipoic acid was determined by K. Mislow and W. C. Meluch, J. Amer. Chem. Soc., **78**, 5920 (1956). The CD spectrum of **I** in dioxane was first reported by C. Djerassi, H. Wolf, and E. Bunnenberg, J. Amer. Chem. Soc., **84**, 4552 (1962). For a review on the "Biochemistry and Chemistry of Lipoic Acid," cf. U. Schmidt, P. Grafen, K. Altland, and H. W. Goedde, Advances in Enzymology, **32**, 423 (1969).

(4) For a treatment of superposed Cotton effects, see K. M. Wellman, P. H. A. Laur, W. S. Briggs, A. Moscowitz, and C. Djerassi, J. Amer. Chem. Soc., **87**, 66 (1965).

(5) I. L. Karle, J. A. Estlin, and K. Britts, Acta Crystallog., **22**, 567 (1967); R. M. Stroud and C. H. Carlisle, Acta Crystallog., Sect. B, **28**, 304 (1972).

(6) O. Foss, A. Hordvik, and J. Sletten, Acta Chem. Scand., **20**, 1169 (1966).

(7) G. Claeson and J. Pedersen, Tetrahedron Lett., 3975 (1968).

(8) M.-O. Hedblom, Tetrahedron Lett., 5159 (1970).

(9) A. F. Beecham, J. W. Loder, and G. B. Russell, Tetrahedron Lett., 1785 (1968).