Chiroptical Properties of Cyclo-L-cystine

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The chiroptical properties associated with the two lowest-energy disulphide transitions in cyclo-L-cystine are calculated on an independent systems model in which both static coupling and dynamic coupling between the dioxopiperazine and disulphide moieties are considered. The structure of cyclo-L-cystine requires that the dihedral angle within the disulphide group be nearly ±90°, with both ***P*** and ***M*** chirality permitted. Special emphasis is placed on calculating the vicinal contributions to disulphide optical activity arising from dissymmetric interactions between the dioxopiperazine and disulphide moieties. Available circular dichroism spectra of cyclo-L-cystine in solution are discussed and interpreted in terms of the calculated results.

MANY studies of disulphide structures have been reported in the literature.[1] The disulphide bond in cystine and cystine derivatives has received special attention due to its important role in maintaining protein structure and, in some cases, its participation in biological reactions. Many spectroscopic methods have been used to probe the conformational and electronic structural features of the disulphide moiety in these systems. Among these various methods, chiroptical spectroscopy is expected to be an especially useful probe due to its extraordinary sensitivity to stereochemical and electronic structural detail in molecular systems. The optical activity and chiroptical properties of various cystine derivatives have been studied extensively.[2,3]

The reliability and accuracy with which optical activity can be used as a probe of molecular stereochemistry and electronic structure depends in large part on the existence of semiempirically based or theoretically derived models which provide explicit spectra–structure relationships. Although these relationships (or rules for making spectra–structure correlations) may be either qualitative or semiquantitative, it is important that they be calibrated against data obtained on compounds of known and preferably rigid structures. Several theoretical studies of disulphide optical activity have been

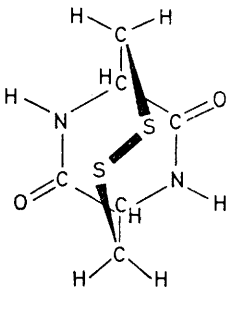
[1] D. B. Boyd, *J. Phys. Chem.*, 1974, **78**, 1554, and references therein.

[2] J. P. Casey and R. B. Martin, *J. Amer. Chem. Soc.*, 1972, **94**, 6141.

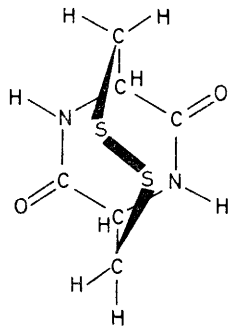
[3] R. W. Strickland, J. Webb, and F. S. Richardson, *Biopolymers*, 1974, **13**, 1269, and references therein.

reported in the literature.[3-6] In three of these studies [4-6] primary attention was focused on the contribution of inherent chirality within the disulphide moiety to the chiroptical observables associated with the near-u.v. transitions of dissymmetric disulphide molecules. Linderberg and Michl [4] and Webb *et al.*[6] adopted the CNDO semiempirical molecular orbital model for calculating the electronic wave functions of several inherently chiral disulphide structures and then used these wave functions to calculate, directly, the electronic rotatory strengths of the near-u.v. disulphide transitions. Woody,[5] on the other hand, based his calculations of disulphide optical activity on the more qualitative Bergson model [7] of the electronic structure and optical properties of disulphide compounds. Strickland *et al.*[3] examined the chiroptical properties of L-cystine conformational isomers on a model in which both inherent disulphide chirality and vicinal effects due to peripheral asymmetric centres were taken into account. In this latter study,[3] vicinal contributions to disulphide optical activity were calculated using the static perturbation (or 'one-electron') model of molecular optical activity.[8-10] Although useful in providing interpretative schemes for much of the available experimental data on chiral disulphide systems, these theoretical studies did not provide definitive results and relationships which could be applied with a high degree of confidence and reliability.

Cyclo-L-cystine (3,4-dithia-7,9-diazabicyclo[4.2.2]decane-8,10-dione) is a particularly attractive model system for examining disulphide optical activity since it has a relatively rigid structure (compared, for example, to the various acyclic cystine derivatives which have been studied). The dioxopiperazine ring in this compound



(Ia) (***P***)



(Ib) (***M***)

has a boat-shaped structure which is bridged by a disulphide group having a dihedral twist angle of *ca.* 90°.[11] Furthermore, based on simple molecular model construction, the dihedral twist angle may be either +90° or −90° so that isomers (Ia) and (Ib) should be nearly isoenergetic.

Although the disulphide moieties in isomers (Ia) and (Ib) are enantiomeric [***P*** chirality in isomer (Ia) and ***M*** chirality in isomer (Ib)], the overall structures of (Ia) and (Ib) are not enantiomeric. Interactions between the disulphide and dioxopiperazine moieties are expected to be different in the two structures leading to slightly different total energies and chiroptical properties for the two isomers. ^1H and ^{13}C N.m.r. studies on cyclo-L-cystine suggest that the ***P*** isomer (Ia) is the more stable of the two.[11] Both structures have exact C_2 symmetry (as drawn above).

The circular dichroism (c.d.) spectrum reported by Donzel *et. al.*[11] for cyclo-L-cystine in ethanol exhibits two extrema in the 185—300 nm region, one centred at 228 nm ($[\theta] = -49\,800°$ cm^2 dmol^{-1}) and one centred at 198 nm ($[\theta] = 296\,000°$ cm^2 dmol^{-1}). The 228 nm band was assigned to a perturbed peptide $n \longrightarrow \pi^*$ transition and the 198 nm band was assumed to arise from a superposition of peptide $\pi \longrightarrow \pi^*$ and disulphide transitions. The absence of any c.d. bands in the 250—300 nm region was taken as evidence of a disulphide moiety with a dihedral twist angle of *ca.* 90° and with two nearly degenerate $n_s \longrightarrow \sigma^*_{ss}$ transitions which make rotatory strength contributions equal in magnitude but opposite in sign. Ignoring vicinal contributions to the long wavelength (250—300 nm) optical activity of the disulphide group, this conclusion is in agreement with predictions made by Linderberg and Michl [4] and by Woody.[5]

Jung and Ottnad [12] have also reported c.d. spectra for cyclo-L-cystine in trifluoroethanol, methanol, and dioxan solvents and as a function of temperature. They too found a negative c.d. band centred near 230 nm and a more intense positive c.d. band centred near 195 nm. The *ca.* 230 nm band was red-shifted on going from trifluoroethanol to methanol to dioxan solvent, and the intensity of this band increased as temperature was lowered over the range 55—0 °C. The temperature dependence of c.d. intensity in this band was cited as evidence for temperature-dependent isomer (Ia) (***P***) ⟷ isomer (Ib) (***M***) interconversion. The (Ia) structure was assumed to be dominant at lower temperatures. In addition to the *ca.* 195 and *ca.* 230 nm bands, Jung and Ottnad also observed a weak positive c.d. band near 305 nm ($[\theta] = 30°$ cm^2 dmol^{-1} in dioxan). Although the intensity of this heretofore unobserved long-wavelength c.d. band was also found to be temperature-dependent, the lack of *strong* temperature-dependence in this band was cited as evidence against its assignment to a disulphide-localized transition. Instead, it was speculated that this band possibly arises from a peptide-disulphide charge-transfer type transition.

Here we re-examine the optical activity associated

[4] J. Linderberg and J. Michl, *J. Amer. Chem. Soc.*, 1970, **92**, 2619.
[5] R. W. Woody, *Tetrahedron*, 1973, **29**, 1273.
[6] J. Webb, R. W. Strickland, and F. S. Richardson, *J. Amer. Chem. Soc.*, 1973, **95**, 4775.
[7] G. Bergson, *Arkiv. Kemi*, 1958, **12**, 233; *ibid.*, 1962, **18**, 409.
[8] E. U. Condon, W. Altar, and H. Eyring, *J. Chem. Phys.*, 1937, **5**, 753.
[9] W. J. Kauzmann, J. E. Walter, and H. Eyring, *Chem. Rev.*, 1940, **26**, 339.
[10] J. A. Schellman, *J. Chem. Phys.*, 1966, **44**, 55.
[11] B. Donzel, B. Kamber, K. Wuthrich, and R. Schwyzer, *Helv. Chim. Acta*, 1972, **55**, 947.
[12] G. Jung and M. Ottnad, *Angew. Chem. Internat. Edn.*, 1974, **13**, 818.

with the two lowest-energy disulphide transitions in cyclo-L-cystine, giving special attention to vicinal contributions arising from disulphide-dioxopiperazine interactions in structures (Ia) and (Ib). Even if the Linderberg–Michl hypothesis is correct and the net long wavelength disulphide optical activity due to inherent disulphide chirality vanishes when the dihedral angle is close to 90°, it does not necessarily follow that vicinal contributions to the long wavelength ($n_s \longrightarrow \sigma^*_{ss}$) optical activity will vanish. In our previous studies of acyclic cystine conformational isomers, we found that vicinal contributions to disulphide optical activity may be substantial.

Methods of Calculation.—To calculate vicinal contributions to the optical activity of the two lowest energy disulphide transitions in isomers (Ia) and (Ib) of cyclo-L-cystine, we adopted an independent systems type model.[13-16] The ground and six lowest-excited states of the disulphide moiety comprise the zeroth-order basis set of chromophoric states in our perturbation model. Electrostatic interactions between the ground state charge distributions on the dioxopiperazine moiety and the chromophoric electron of the disulphide group result in a mixing or scrambling of these states in the spirit of the static coupling (or ' one-electron ') theory of molecular optical activity. The disulphide group wave functions were calculated in the CNDO approximation [17] and excited states were constructed in the virtual orbital-configuration interaction approximation.[3,6] Ground state charge distributions on the dioxopiperazine moiety were represented by point charges taken from atomic charge densities computed in a previous study on dioxopiperazine structures.[18]

Electrostatic interactions between transition densities associated with $\pi \longrightarrow \pi^*$ transitions in the dioxopiperazine moiety and transition densities associated with disulphide transitions were also calculated. These interactions couple the transition moments associated with transitions localized in various parts (or groups) of the molecule and lead to the so-called dynamic coupling contributions to optical activity.[13] Only interactions between the two lowest-energy (and nearly degenerate) $\pi \longrightarrow \pi^*$ transitions of the dioxopiperazine moiety and the two lowest-energy (and nearly degenerate) $n_s \longrightarrow \sigma^*_{ss}$ transitions of the disulphide chromophore were included in our dynamical coupling calculations. Both the $\pi \longrightarrow \pi^*$ dioxopiperazine transition densities and the $n_s \longrightarrow \sigma^*_{ss}$ disulphide transition densities were obtained from previous calculations on model systems.[3,18] Only electric dipole–electric dipole interaction terms were included in our dynamic coupling calculations.

Vicinal contributions to the $n_s \longrightarrow \sigma^*_{ss}$ rotatory strengths were calculated for both structures (Ia) and (Ib), assuming a dihedral twist of |90°| in each case.

RESULTS

The two lowest-energy disulphide transitions are both calculated to be of the $n_s \longrightarrow \sigma^*_{ss}$ type and are nearly degenerate (290 and 288 nm). The lower-energy transition (290 nm) has *B* symmetry and is polarized perpendicular to the C_2 symmetry axis. The higher-energy member of the couplet (288 nm) has *A* symmetry and is polarized parallel to the C_2 symmetry axis. Since the dioxopiperazine $\pi \longrightarrow \pi^*$ transitions are polarized perpendicular to the C_2 axis in each structure, (Ia) and (Ib), they do not couple to the $n_s \longrightarrow \sigma^*_{ss}$ (*A*) disulphide transition. Dynamic coupling occurs only between the $n_s \longrightarrow \sigma^*_{ss}$ (*B*) and $\pi \longrightarrow \pi^*$ transitions in the electric dipole–electric dipole approximation. Both the $n_s \longrightarrow \sigma^*_{ss}$ (*A*) and $n_s \longrightarrow \sigma^*_{ss}$ (*B*) disulphide transitions are perturbed by the static coupling interaction terms in our model.

The total rotatory strengths induced in the disulphide $n_s \longrightarrow \sigma^*_{ss}$ (*A*) and $n_s \longrightarrow \sigma^*_{ss}$ (*B*) transitions by static and dynamic disulphide-dioxopiperazine interactions were calculated to be:

	(Ia) (***P***)	(Ib) (***M***)
$R(n_s \longrightarrow \sigma^*_{ss}, A)$	4.25×10^{-40} e.s.u.^{2} cm^2	-4.28×10^{-40} e.s.u.^{2} cm^2
$R(n_s \longrightarrow \sigma^*_{ss}, B)$	-3.60×10^{-40} e.s.u.^{2} cm^2	3.65×10^{-40} e.s.u.^{2} cm^2
R(net)	0.65×10^{-40} e.s.u.^{2} cm^2	-0.63×10^{-40} e.s.u.^{2} cm^2

where $R(\text{net}) = R(n_s \longrightarrow \sigma^*_{ss}, A) + R(n_s \longrightarrow \sigma^*_{ss}, B)$.

DISCUSSION

The results presented in the previous section show that vicinal perturbations of the disulphide chromophore by the dioxopiperazine moiety in isomer (Ia) (***P***) should induce a small positive *net* rotatory strength in the long-wavelength $n_s \longrightarrow \sigma^*_{ss}$ (*A*) and $n_s \longrightarrow \sigma^*_{ss}$ (*B*) transitions. Vicinal perturbations of the disulphide chromophore by the dioxopiperazine group in isomer (Ib) (***M***) lead to a small negative *net* rotatory strength in these disulphide transitions. The magnitudes of the induced net rotatory strengths in isomers (Ia) and (Ib) are nearly the same.* The magnitudes calculated for these ' vicinal ' contributions to $n_s \longrightarrow \sigma^*_{ss}$ rotatory strength are very small compared to those calculated from the observed spectra for the *ca.* 230 nm c.d. band and the *ca.* 195 nm band.[11] Additionally, they are much smaller than those expected to arise from inherent chirality within the disulphide moiety. If, however, it is assumed along with Linderberg and Michl [4] and with Woody [5] that the *net* rotatory strength contributed by inherent chirality to the $n_s \longrightarrow \sigma^*_{ss}$ transitions is essentially zero in structures such as (Ia) and (Ib), then the computed ' vicinal ' or

* The very small difference in *net* rotatory strengths (0.02×10^{-40} e.s.u.^{2} cm^2) calculated for the isomers (Ia) (***P***) and (Ib) (***M***) must be considered only marginally significant given the very approximate nature of the theoretical model used. That is, given the approximations inherent in our calculations the absolute magnitudes of R(net) calculated for the (Ia) (***P***) and (Ib) (***M***) isomers are essentially the same.

[13] E. G. Höhn and O. E. Weigang, *J. Chem. Phys.*, 1968, **48**, 1127.
[14] P. M. Bayley, E. B. Nielsen, and J. A. Schellman, *J. Phys. Chem.*, 1969, **73**, 228.
[15] R. Nagarajan and R. W. Woody, *J. Amer. Chem. Soc.*, 1973, **95**, 7212.
[16] J. A. Schellman and P. Oriel, *J. Chem. Phys.*, 1962, **37**, 2114.
[17] J. A. Pople and D. L. Beveridge, 'Approximate Molecular Orbital Theory,' McGraw Hill, New York, 1970.
[18] F. S. Richardson and W. Pitts, *Biopolymers*, 1974, **13**, 703.

induced $n_S \longrightarrow \sigma^*_{SS}$ rotatory strengths can be assumed to account for the very weak positive c.d. band observed by Jung and Ottnad [12] near 305 nm for cyclo-L-cystine. The c.d. bond at 305 nm is somewhat to the red of the region where disulphide $n_S \longrightarrow \sigma^*_{SS}$ transitions are expected to occur when the dihedral twist angle is *ca.* 90° (experimentally, these transitions are generally found near 250—260 nm in acyclic disulphides with dihedral angles of *ca.* 90°). However, it is entirely possible that the disulphide–dioxopiperazine interactions will significantly shift these transitions to the red in cyclo-L-cystine. Furthermore, the relatively strong negative c.d. band centred near 230 nm is quite broad and it is possible that its long negative tail in the 230—280 nm region may result in a red-shift of the apparent maximum of the long wavelength positive c.d. band observed by Jung and Ottnad.[12] In any case it seems unlikely that this weak positive c.d. band near 305 nm must be assigned to something other than a disulphide $n_S \longrightarrow \sigma^*_{SS}$ transition (such as a charge-transfer transition as suggested by Jung and Ottnad). The positive net rotatory strength calculated for the (Ia) (***P***) isomer further supports the proposal made by Donzel *et. al.*[11] that this isomer is the more stable of the two [(Ia) and (Ib)].

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