The Circular Dichroism of Gliotoxin and Related Epidithiapiperazinediones

R. Nagarajan[1a] **and Robert W. Woody*** [1b]

Contribution from the Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46206, and the Department of Chemistry, Arizona State University, Tempe, Arizona 85281. *Received June 16, 1973*

Abstract: The circular dichroism spectra of gliotoxin and related antibiotics are complex. The CD of gliotoxin has been the subject of some controversy, since it represents a violation of the skewed diene rule. We report here new measurements of the circular dichroism spectra of the epidithiapiperazinedione antibiotics gliotoxin (**1**), acetylaranotin (**2b**), acetylapoaranotin (**3b**), sporidesmin (**4**), and di-*O*-acetyl chaetocin (**5**). Various partially reduced dethio derivatives of **2b** are also presented. A theoretical analysis of the CD of these epidithiapiperazinediones gives a good account of the long-wavelength transitions. The CD bands of **1** are assigned as follows: (1) 340 nm, disulfide $n\sigma^*$ transition; (2) 310 nm, $n_1 \rightarrow \pi^*$ charge-transfer band, where n_1 is the highest filled orbital of the disulfide and π^* represents the antibonding π orbitals of the peptide groups; (3) 270 nm, overlapping $\pi-\pi^*$ transition in the diene group and n_2, $n_3 \rightarrow \pi^*$ charge-transfer bands, where n_2 and n_3 are disulfide orbitals; (4) 235-nm band, overlapping of peptide $n\pi^*$ transitions and disulfide n_2, $n_3 \rightarrow \sigma^*$ transitions. In the CD spectrum of **2b**, the dihydrooxepine rings contribute to a CD band at 230 nm; the 270-nm band is due to the charge-transfer bands alone, while the other bands coincide with those in **1**. The positive 270-nm band of gliotoxin represents an apparent violation of the skewed diene rule, since the diene has a left-handed twist. Our results show that this anomaly results from two factors: the presence of a positive charge-transfer band at *ca.* 270 nm, and coupling of the diene $\pi\pi^*$ transition with a disulfide $n\sigma^*$ transition. In sporidesmin and chaetocin, the sign of the long-wavelength disulfide transition does not obey the usual disulfide chirality rule. Our calculations suggest that this is due to coupling with the peptide $\pi\pi^*$ transitions and perhaps with the indolinyl chromophores. Finally, we suggest that the sign of the charge-transfer bands at 270 and 310–320 nm in epidithiapiperazinedione systems represents a useful criterion of absolute configuration.

The epidithiapiperazinedione group is the nucleus of an important class of fungal metabolites which are of great pharmacological and chemical interest. This group of antibiotics includes gliotoxin[2] (**1** of Figure 1), sporidesmin[3] (**4**), aranotin[4] (**2a**), chaetocin[5] (**5**), and chaetomin.[6] All of these compounds inhibit bacterial growth[7] and many of them are inhibitors of viral multiplication in tissue cultures.[8,9] They are, however, rather toxic to mammalian cells themselves[9] so that their therapeutic value has been limited.

The close juxtaposition of various chromophoric groups in these molecules makes their electronic spectra complex and challenging to interpret. However, the additional complexity may yield important information. Gliotoxin, the first representative of this class of molecules to be recognized,[2] presents a case in point. The circular dichroism of gliotoxin was first reported by Beecham and Mathieson.[10] They observed a strong positive CD band at 270 nm which they very reasonably assigned to the long-wavelength $\pi-\pi^*$ transition of the 1,3-cyclohexadiene ring. As Beecham and Mathieson noted, the positive sign for this band is a violation of the skewed diene rule,[11] which states that for a cisoid diene skewed in a right-handed screw sense the long-wavelength band will be positive, while a left-handed diene will have a negative CD band at long wavelengths. In gliotoxin, the group has a *left-handed* screw sense[12,13] and hence one would expect a *negative* 270-nm band rather than the observed *positive* band.

Ziffer, *et al.*,[14] proposed that the apparent violation of the skewed diene rule in gliotoxin results from a strong interaction of the diene and the disulfide. In support of this, they showed that in dethiogliotoxin, in which the disulfide has been removed, there is a strong negative band at 265 nm, in agreement with the skewed diene rule, assuming that no major conformational (or configurational) changes occur on disulfide removal.

The closely related molecule, aranotin, has a CD spectrum remarkably similar to that of gliotoxin.[15] In particular, there is a positive band at 265 nm comparable in magnitude to that of gliotoxin. This seems surprising in view of the fact that the conjugated diene

(1) (a) Eli Lilly and Company; (b) Arizona State University.

(2) M. R. Bell, J. R. Johnson, B. S. Wildi, and R. B. Woodward, *J. Amer. Chem. Soc.*, 80, 1001 (1958).

(3) (a) R. Hodges, J. W. Ronaldson, A. Taylor, and E. P. White, *Chem. Ind. (London)*, 42 (1963); (b) J. Fridrichsons and A. McL. Mathieson, *Acta Crystallogr.*, 18, 1043 (1965).

(4) (a) R. Nagarajan, L. L. Huckstep, D. H. Lively, D. C. De Long, M. M. Marsh, and N. Neuss, *J. Amer. Chem. Soc.*, 90, 2980 (1968); (b) P. A. Miller, P. W. Trown, W. Fulmor, G. O. Morton, and J. Karliner, *Biochem. Biophys. Res. Commun.*, 33, 219 (1968).

(5) (a) D. Hauser, H. P. Weber, and H. P. Sigg, *Helv. Chim. Acta*, 53, 1061 (1970). (b) The representation of chaetocin in **5** is not the same as that given in ref 5a but is consistent with the text of that paper. The two-dimensional drawing in ref 5a is actually that of a meso form. We are grateful to Dr. Noel Jones of Eli Lilly and Company for drawing our attention to this.

(6) S. Safe and A. Taylor, *J. Chem. Soc., Perkin Trans. 1*, 472 (1972).

(7) D. Brewer, D. E. Hannah, and A. Taylor, *Can. J. Microbiol.*, 12, 1187 (1966).

(8) W. A. Rightsel, H. G. Schneider, B. J. Sloan, P. R. Graf, F. A. Miller, Q. R. Bartz, J. Ehrlich, and G. J. Dixon, *Nature (London)*, 204, 1333 (1964).

(9) For a review of biological activities of this class of antibiotics, see A. Taylor, "Biochemistry of Some Foodborne Microbial Toxins," R. I. Mateles and G. N. Wogan, Ed., MIT Press, Cambridge, Mass., 1967, p 69.

(10) A. F. Beecham and A. McL. Mathieson, *Tetrahedron Lett.*, 3139 (1966).

(11) A. Moscowitz, E. Charney, U. Weiss, and H. Ziffer, *J. Amer. Chem. Soc.*, 83, 4661 (1961).

(12) A. F. Beecham, J. Fridrichsons, and A. McL. Mathieson, *Tetrahedron Lett.*, 3131 (1966).

(13) J. Fridrichsons and A. McL. Mathieson, *Acta Crystallogr.*, 23, 439 (1967).

(14) H. Ziffer, U. Weiss, and E. Charney, *Tetrahedron*, 23, 3881 (1967).

(15) R. Nagarajan, N. Neuss, and M. M. Marsh, *J. Amer. Chem. Soc.*, 90, 6518 (1968).

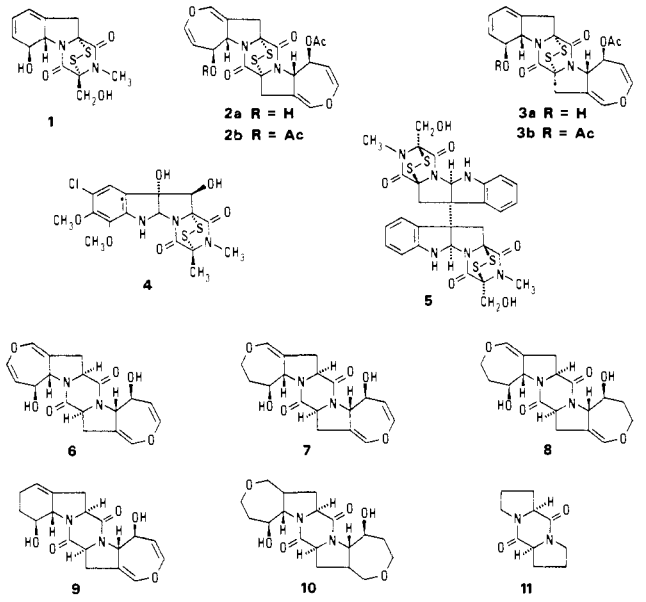

Figure 1. Structures of epidithiapiperazinediones and their derivatives.

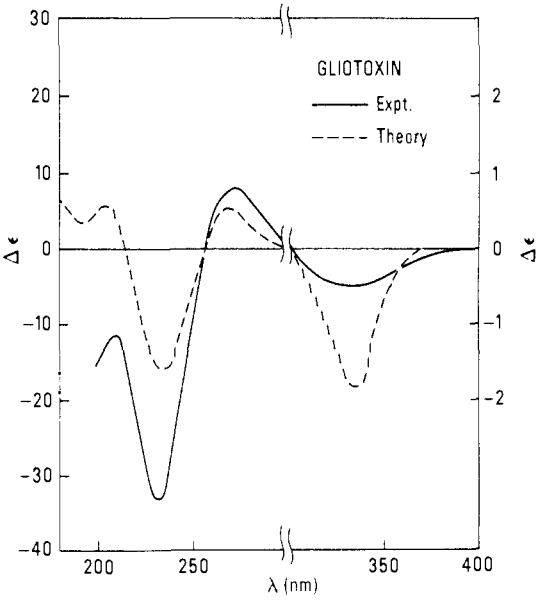

Figure 2. Theoretical (- - -) and experimental (—) CD curves of gliotoxin (**1**).

chromophore of gliotoxin is absent in aranotin and is replaced by two dihydrooxepine or divinyl ether chromophores, which would not be expected to have any absorption bands in the 250–260-nm region.

Table I. Experimental Circular Dichroism Parameters for Gliotoxin and Acetylaranotin[a]

Gliotoxin				Acetylaranotin			
λ_{max}, nm	$\Delta\epsilon_{max}$	Δ,[b] nm	R,[c] DBM	λ_{max}, nm	$\Delta\epsilon_{max}$	Δ,[b] nm	R,[c] DBM
338	−0.49	32	−0.021	344	−0.67	27	−0.023
312	−0.19	10	−0.003	307	−0.23	15	−0.005
272	+7.4	22	+0.257	262	+9.0	18	+0.272
234	−33.5	15	−0.946	228	−72.0	18	−2.524

[a] In methanol. [b] Δ is half the width of the Gaussian band at the point where the ordinates have fallen to $1/e$ of the maximum. [c] The rotational strength is expressed in Debye Bohr magnetons (1 DBM = 0.9273×10^{-38} cgs unit).

In this paper we will report experimental and theoretical studies of the CD of gliotoxin, aranotin, and a number of their derivatives. This work has led us to a rather complete picture of the low-energy transitions of the epidithiapiperazinedione chromophore. This picture will be useful in interpreting the CD of more complex molecules such as sporidesmin, chaetocin, etc. In particular, we have found a new criterion of absolute configuration in these systems which correctly correlates the CD with configuration in all systems known so far. We have also found an explanation for the breakdown of the skewed diene rule in gliotoxin.

Experimental Section

Methods. The circular dichroism spectra were determined in a Cary Model 6001 circular dichroism accessory mounted on the Cary 60 spectropolarimeter, and the ultraviolet spectra were determined in a Cary Model 15 spectrophotometer. The concentration of the compounds used for measuring the spectra was between 10^{-4} and 10^{-5} *M*. The experimental CD spectra of gliotoxin and acetylaranotin were resolved into Gaussian compounds using a Du Pont curve resolver.

All the new compounds described gave the expected composition

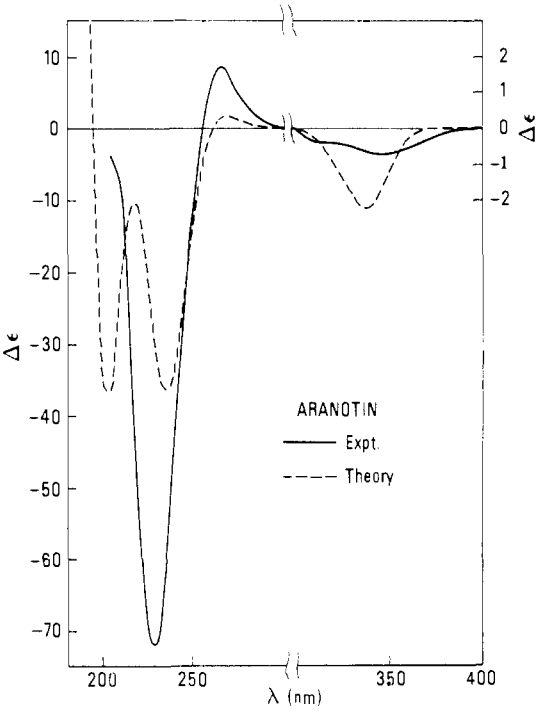

Figure 3. Theoretical (- - -) and experimental (—) CD curves of acetylaranotin (**2b**).

either by elemental analysis or high resolution mass spectra. In addition, their nmr, infrared, ultraviolet, and mass spectra were consistent with their structures.

Results. The CD curves for gliotoxin and acetylaranotin, determined in methanol, are shown in Figures 2 and 3, respectively. The position, amplitude, and half-width from the Gaussian band analysis and the rotational strengths calculated from these parameters are given in Table I.

The CD spectra of gliotoxin and acetylaranotin were also measured in trifluoroethanol, acetonitrile, and dioxane. The position and amplitudes of the extrema in the various solvents are listed in Table II.

Figures 4 and 5 show respectively the absorption and CD spectra of sporidesmin (**4**) and di-*O*-acetyl chaetocin (diacetyl **5**), determined in methanol. The CD spectra of several partially reduced and desulfurized derivatives of aranotin (**6**, **7**, and **8**), determined in

Table II. Solvent Effects on the CD of Gliotoxin and Acetylaranotin

Solvent	Maxima in nm (Δε)									
	Gliotoxin					Acetylaranotin				
CF_3CH_2OH	335 (−0.33)	318 (−0.26)	269 (+8.5)	230 (−31.4)	195 (−20.4)	343 (−0.50)	310[a] (−0.12)	267 (+8.0)	227 (−75.2)	202[a] (−10.5)
CH_3OH	337 (−0.46)	322[a] (−0.44)	271 (+7.5)	233 (−31.2)	198 (−13.3)	344 (−0.67)	310[a] (−0.33)	266 (+8.2)	228 (−72.0)	205[a] (−4.1)
CH_3CN	336 (−0.47)	320[a] (−0.45)	270 (+6.6)	234 (−30.5)	200 (−16.5)	345 (−0.71)	310[a] (−0.41)	265 (+8.2)	228 (−71.5)	205[a] (−9.8)
Dioxane	337 (−0.51)	320[a] (−0.44)	271 (+6.6)	235 (−29.3)		347 (−0.81)	313[a] (−0.41)	265 (+8.2)	228 (−73.9)	

[a] Inflexion.

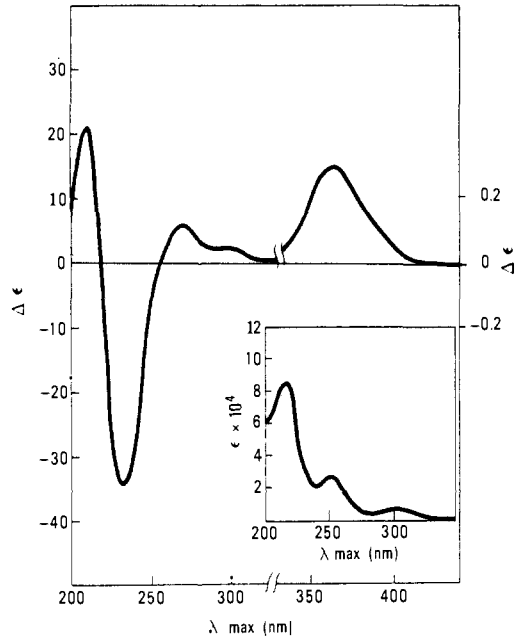

Figure 4. Uv and CD spectra of sporidesmin (**4**) in MeOH.

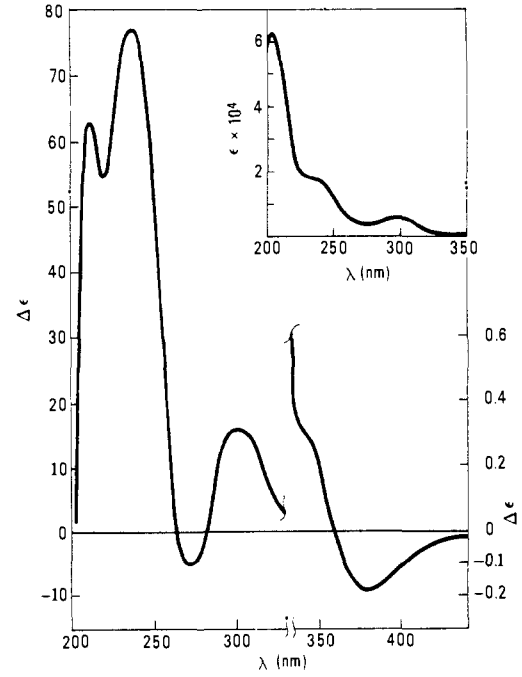

Figure 5. Uv and CD spectra of di-*O*-acetylchaetocin (**5**) in MeOH.

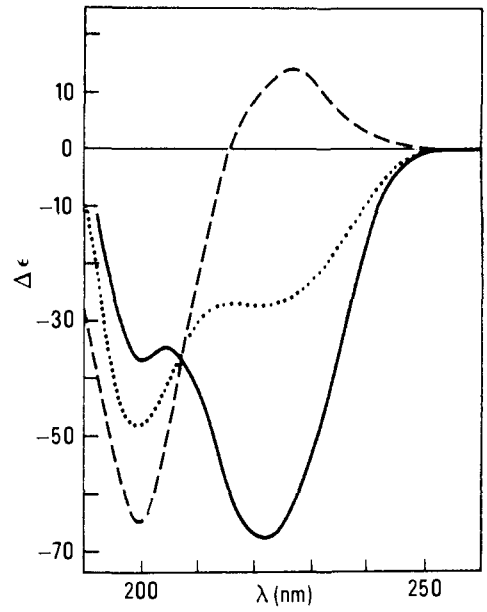

Figure 6. CD spectra of dethiodeacetylaranotin (**6**) (—), dihydrodethiodeacetylaranotin (**7**) (·····), and tetrahydrodethiodeacetylaranotin (**8**) (- - -) in MeOH.

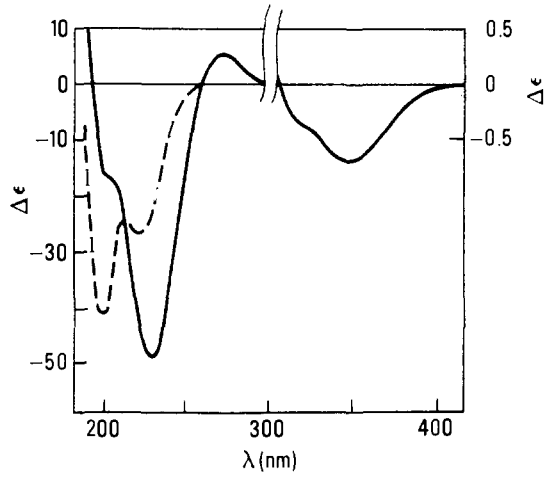

Figure 7. CD spectra of acetylapoaranotin (**3b**) (—) and dihydrodethiodeacetylapoaranotin (**9**) (- - -) in MeOH.

methanol, are given in Figure 6. Figure 7 gives the CD spectra of acetylapoaranotin (**3b**) and of **9** determined in methanol. In Figure 8, we give the CD spectra of octahydrodethiodeacetylaranotin (**10**) and of L-prolyl-L-proline diketopiperazine (**11**), taken in aqueous solution.

Methods

Theoretical. The structure of gliotoxin was taken from the X-ray work of Fridrichsons and Mathie-

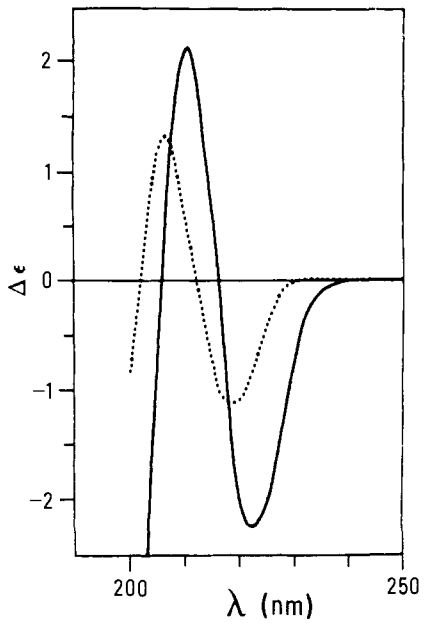

Figure 8. CD spectra of octahydrodethiodeacetylaranotin (**10**) (—) and L-prolyl-L-proline diketopiperazine (**11**) (- - -) in water.

son.[13] The coordinates which they reported refer to a left-handed coordinate system, whereas we use a right-handed system. Thus, their coordinates were first converted from the monoclinic axis system to a Cartesian coordinate system and then the z coordinate reversed in sign. There are two molecules per unit cell with somewhat different geometries. We used the coordinates for molecule B (notation of ref 13) because the coordinates for N2 in molecule A appeared to contain an error. Although the epidithiapiperazinedione ring of gliotoxin does not have an exact twofold axis, there is an approximate C_2 axis perpendicular to the disulfide bond and passing through its midpoint. To simplify the geometric problem, such an axis was assumed in the calculations by generating one of the amide groups from the other by a C_2 operation.

The X-ray structure of acetylaranotin has been reported in part,[16] but the detailed coordinates have not been published. The structure of aranotin was therefore assumed to contain a epidithiapiperazinedione nucleus and a prolyl ring identical with that of gliotoxin. One of the double bonds of the dihydrooxepine ring therefore coincides with the 5,6 double bond of gliotoxin. The position of the other double bond was deduced from Dreiding models. Two alternative conformations[15] for the dihydrooxepine ring, illustrated in Figure 9, were considered. These are denoted by conformations A and B. The other prolyl and dihydrooxepine rings were generated by carrying out a C_2 operation about the assumed twofold axis. Cosulich, *et al.*,[16] specifically state that the conformation of the aranotin nucleus differs appreciably from that of gliotoxin. However, they do not give any detailed differences and a comparison of the reported bond lengths and angles for gliotoxin and aranotin shows that the differences in these parameters are not very great. We have therefore ignored the differences between the two structures.

(16) D. B. Cosulich, N. R. Nelson, and J. H. van den Hende, *J. Amer. Chem. Soc.*, **90**, 6519 (1968).

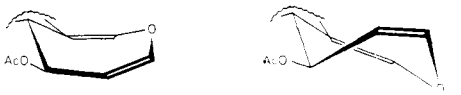

Figure 9. Conformations of dihydrooxepin ring in acetylaranotin (**2b**).

The structures of various derivatives of gliotoxin and aranotin, such as the dethio derivatives, those in which one or more double bonds are saturated, etc., were treated assuming that the diketopiperazine nucleus remains unchanged from that of gliotoxin.

In calculating the rotational strengths of the various localized transitions (those within individual chromophores), the approach used was like that used by Bayley, *et al.*,[17] and by Chen and Woody[18] in treating polypeptide systems, except that the dipole velocity operator[19] was used rather than the dipole length.[17,18]

The individual chromophores were treated as follows.

(a) The Peptide Groups. The peptide $n\pi^*$ and $\pi\pi^*$ transitions were considered, using transition monopole positions and charges previously developed for polypeptides.[20] The wavelength of the unperturbed $\pi\pi^*$ transition was taken to be 200 nm, appropriate for a tertiary amide according to Nielsen and Schellman.[21] The $n\pi^*$ transition was located at 232 nm, a position consistent with the data of Nielsen and Schellman for nonhydrogen-bonded amides and also consistent with the strong CD band observed for gliotoxin.[15] The dipole velocity operator for the $\pi\pi^*$ transition was taken to have a magnitude of 0.5017 $Å^{-1}$, calculated from the oscillator strength reported by Peterson and Simpson[22] for myristamide, and the direction of the dipole velocity operator was determined from the observed polarization direction for myristamide.[22]

(b) The Disulfide Group. Four $n \rightarrow \sigma^*$ transitions in the disulfide group were included in the calculation. Monopole charges, transition dipole velocities, and magnetic dipole transition moments for these transitions were derived from the general equations presented elsewhere.[23] The dihedral angle used was $\varphi = -15.8°$.[13] The energies for these disulfide transitions correspond to the following wavelengths: $n_1 \rightarrow \sigma^*$, λ_1 337 nm; $n_2 \rightarrow \sigma^*$, 235 nm; $n_3 \rightarrow \sigma^*$, 235 nm; and $n_4 \rightarrow \sigma^*$, 200 nm. Of these transitions, the only one for which we have unambiguous evidence is the 337-nm band which corresponds to the long-wavelength band in the gliotoxin spectrum.[15] The location of the other three bands is considerably more speculative but is based on the following considerations. The disulfide model which we have described previously[23] predicts four evenly spaced $n\sigma^*$ transitions which for dihedral angles of 10–20° would run from *ca.* 340 nm down to *ca.* 200 nm. The INDO-type calculations of Yamabe, *et al.*,[24]

(17) P. M. Bayley, E. B. Nielsen, and J. A. Schellman, *J. Phys. Chem.*, 73, 228 (1969).
(18) A. K. Chen and R. W. Woody, *J. Amer. Chem. Soc.*, **93**, 29 (1971).
(19) A. Moscowitz, Ph.D. Thesis, Harvard University, 1957.
(20) R. W. Woody, *J. Chem. Phys.*, **49**, 4797 (1968).
(21) E. B. Nielsen and J. A. Schellman, *J. Phys. Chem.*, 71, 2297 (1967).
(22) D. L. Peterson and W. T. Simpson, *J. Amer. Chem. Soc.*, 77, 3929 (1955).
(23) R. W. Woody, *Tetrahedron*, **29**, 1273 (1973).
(24) H. Yamabe, H. Kato, and T. Yonezawa, *Bull. Chem. Soc. Jap.*, **44**, 604 (1971).

on $(CH_3)_2S_2$ and similar systems suggest that for small dihedral angles, the lowest and highest energy n → σ* transitions ($n_1 \rightarrow \sigma^*$ and $n_4 \rightarrow \sigma^*$) should be located approximately as in the simple theory. However, Yamabe, *et al.*,'s calculations indicate that the $n_2 \rightarrow \sigma^*$ and $n_3 \rightarrow \sigma^*$ transitions should be closely spaced and should occur at about 1.5 eV above the lowest energy transition. This would place these transitions in the 230–240-nm region, coincident with the very strong CD band at 235 nm in gliotoxin.[15]

(c) The Diene Group. The four π–π* transitions of the diene group were treated using wave functions obtained from a PPP[25] calculation on butadiene skewed at the dihedral angle of −12.5° observed in gliotoxin.[13] Configuration interaction among the four ππ* configurations was taken into consideration and the transition monopoles, dipole velocities, and magnetic moments were calculated from the CI wave functions. This calculation located the lowest energy transition for the skewed butadiene at 5.3 eV (234 nm). The cyclohexadiene ring system of gliotoxin typically has its low-energy ππ* transition at 4.6–4.8 eV (260–270 nm).[26] The first ππ* transition was located at 270 nm and the predicted energies of the other transitions were adjusted downwards by the same amount (0.7 eV).

(d) The Divinyl Ether Group. The divinyl ether chromophore of aranotin was treated as if it consisted of two separate ethylene chromophores. A single π–π* transition was considered in each chromophore with transition monopoles and dipole velocity given by theory. The energy of the individual ππ* transitions was assigned as 5.9 eV. Interaction between the chromophores leads to two exciton components, the one at lower energy being located at ~230 nm, in agreement with the absorption spectrum of aranotin which shows a definite shoulder at 230 nm.[15]

(e) Isolated Ethylenic Chromophores. In various partially reduced derivatives of gliotoxin, aranotin, and apoaranotin, we encounter trisubstituted ethylene or vinyl ether chromophores. These were treated using the same parameters as for the individual double bonds of the divinyl ether chromophore (d), except that the energy of the trisubstituted ethylene ππ* transition was assigned as 6.4 eV (λ_{max} ~195 nm), from data on cyclohexene derivatives.[27]

The rotational strengths of the charge-transfer transitions between the disulfide chromophore and the peptide chromophore were calculated from the transition dipole velocity and magnetic moment matrix elements. Evaluation of these matrix elements is described in the Appendix. The transitions considered were those in which the donor orbital was one of the four lone-pair orbitals of the disulfide (n_1, n_2, n_3, n_4) and the acceptor orbital was the π* orbital of a peptide group. Charge-transfer transitions in the opposite direction (n → σ* and π → σ*, where n and π are the highest filled amide orbitals, and σ* is the antibonding σ orbital of the disulfide group) were considered briefly but their calculated rotational strengths turned out to be negligible.

Circular dichroism curves to be compared with experiment were calculated assuming Gaussian bands. For simplicity, it was assumed that each CD band had the same shape, with a half-width of 15 nm at $1/e$ of the maximum.

Results

Theoretical. The rotational strengths calculated for gliotoxin are given in Table III and compared with the experimental rotational strengths. The last column of Table III gives the assignment of the theoretical CD bands. These assignments represent the dominant contribution in each case. Mixing of electronic transitions become pronounced in the higher energy transitions but in general there is no difficulty in making these assignments. The experimental 270- and 235-nm bands are believed to be made up of two or more different transitions as indicated in Table III. No attempt was made to resolve these contributions in the experimental spectra.

Table III. Theoretical Rotational Strengths for Gliotoxin and Acetylaranotin

λ_{max}, nm	R_{calcd}, DBM	R_{net}, DBM	R_{exptl}, DBM	Assignment
		Gliotoxin		
338	−0.030		−0.021	Disulfide $n_1 \rightarrow \sigma^*$
322	−0.016		−0.003	Charge transfer
273	+0.076	+0.135	+0.257	Diene π → π*
265	+0.059			Charge transfer
236	−0.285			Disulfide n_2, $n_3 \rightarrow \sigma^*$
235	+0.028	−0.479	−0.946	Charge transfer
233	−0.127			DKP n → π*
229	−0.095			Disulfide n_2, $n_3 \rightarrow \sigma^*$
206	+0.166			Disulfide $n_4 \rightarrow \sigma^*$
202	+0.113			DKP π → π*
198	−0.042			DKP π → π*
197	−0.052			DKP π → π*
191	−0.040			Diene π → π*
177	+0.282			Diene π → π*
151	−0.128			Diene π → π*
		Aranotin		
339	−0.038		−0.023	Disulfide $n_1 \rightarrow \sigma^*$
322	−0.016		−0.005	Charge transfer
265	+0.059		+0.272	Charge transfer
240	−0.400	−1.042	−2.524	Disulfide n_2, $n_3 \rightarrow \sigma^*$
235	+0.028			Charge transfer
233	−0.133			DKP n → π*
233	−0.088			DKP n → π*
231	−0.269			DVE π → π*
230	−0.499			DVE π → π*
226	+0.319			Disulfide n_2, $n_3 \rightarrow \sigma^*$
207	+0.454			Disulfide $n_4 \rightarrow \sigma^*$
203	+4.294			DKP π → π*
200	−9.010			DKP π → π*
197	−0.052			DKP π → π*
192	+1.585			DVE π → π*
191	+3.447			DVE π → π*

Figure 2 shows a comparison of the calculated CD curve with the experimental curve for gliotoxin.[15] The calculated CD curve shows qualitative agreement with experiment at wavelengths above about 220 nm. Below that wavelength, the theory fails to reproduce one or more shorter wavelength negative band(s) which have not been completely characterized experimentally. From Table III, we see that, in general, the calculated rotational strengths agree within a factor of 2 with experiment. The only exception is the 312-nm band which we have assigned as an intramolecular charge-transfer transition. Here the calculated rotational strength is

(25) J. N. Murrell, "Theory of the Electronic Spectra of Organic Molecules," Wiley, New York, N. Y., 1963, p 108 ff.
(26) A. I. Scott, "Interpretation of the Ultraviolet Spectra of Natural Products," Macmillan, New York, N. Y., 1964, pp 48 and 49.
(27) Reference 26, pp 20 and 21.

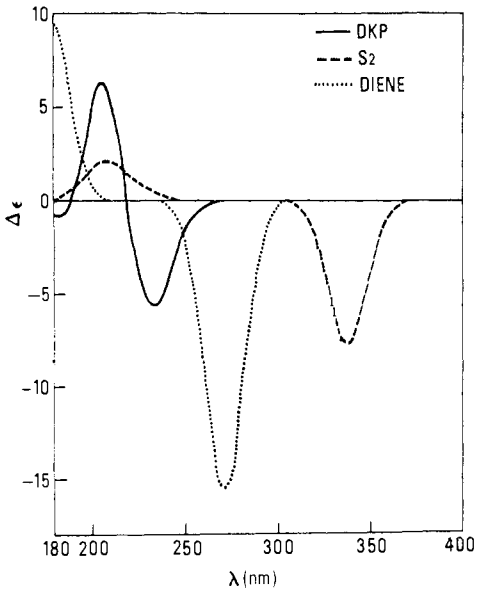

Figure 10. Theoretical CD spectra for isolated chromophoric groups of gliotoxin: diketopiperazine group (—); disulfide (- - -); diene (· · · · · ·).

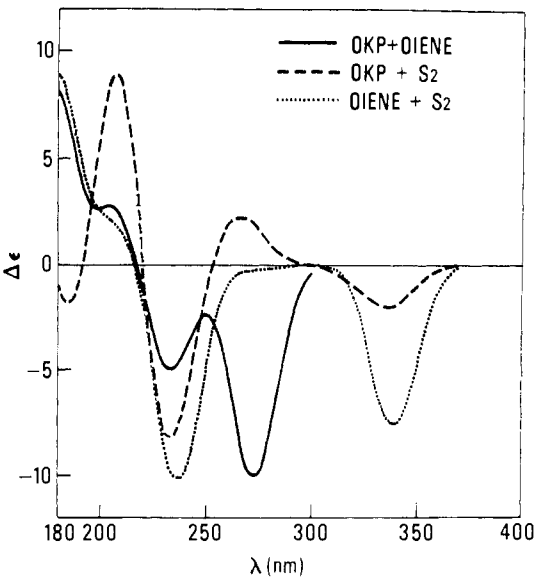

Figure 11. Theoretical CD spectra for pairs of interacting chromophores in gliotoxin: diketopiperazine + diene (—); diketopiperazine + disulfide (- - -); diene + disulfide (· · · · · ·).

too large by a factor of *ca.* 5. This band, however, is not clearly resolved experimentally and so is subject to some uncertainty. Moreover, the calculated rotational strengths of the charge-transfer bands are no doubt much more uncertain than those of the localized transitions.

We have also carried out calculations for various pairwise combinations of the three chromophores and for the isolated chromophores of gliotoxin. The calculated CD curves for these systems are shown in Figures 10 and 11. These results will be discussed further below and compared with the fragmentary data available. Here we shall simply point out that a comparison of these curves with the results for gliotoxin itself (Figure 2) shows striking evidence of the strong interactions among the chromophores. The most evident and important example of this interaction is the strong deviation from additivity of the disulfide and diene chromophores which can be seen by comparing the curves in Figure 10 for the isolated chromophores with the CD curve in Figure 11 for the diene + disulfide pair. The strong negative 270-nm band of the isolated diene is reduced to a very weak negative band when the diene and disulfide are considered together. Finally, in gliotoxin, this band is positive when all three chromophores are taken into consideration.

The calculated rotational strengths for aranotin are also compared with experimental data in Table III. We have presented calculated properties for only one of the two conformations (Figure 9) of the dihydrooxepine ring which we considered (see Methods). Conformation B gave significantly better agreement with experiment, although the differences are only quantitative so we cannot rule out the possible presence of the other conformation in the seven-membered rings. A comparison of the calculated CD curve for aranotin with the experimental curve[15] is shown in Figure 3. Again, the agreement with experiment is qualitatively good in the long-wavelength region ($\lambda > ca.$ 210 nm).

In Table IV, we present calculated rotational strengths for various aranotin derivatives (dethiodeacetylaranotin (**6**)) and derivatives in which one or both seven-membered rings of dethiodeacetylaranotin have been half-reduced (**7** and **8**).

Table IV. Theoretical Calculations on Derivatives of Aranotin and Apoaranotin

λ_{max}, nm	*R*, DBM	λ_{max}, nm	*R*, DBM	λ_{max}, nm	*R*, DBM
6		**7**		**8**	
233	−0.202	233	−0.078	233	−0.012
231	−0.031	231	−0.179	216	−1.030
230	−0.614	215	−1.168	213	+0.062
203	+4.669	202	−0.038	197	−0.105
200	−8.422	195	−0.366	195	+0.987
192	+0.890	192	+1.652		
191	+3.464				
3		Dethio **3**		**9**	
339	−0.023	273	−0.462	233	−0.080
322	−0.016	233	−0.193	231	−0.356
273	−0.075	231	−0.568	205	+0.298
265	+0.059	202	+0.851	201	−2.368
242	−0.319	199	−1.670	192	+1.852
235	+0.028	192	+1.897	189	+0.480
233	−0.236	191	−0.021		
231	−0.846	177	+0.005		
224	+0.120	151	−0.128		
207	+0.293				
202	+0.657				
198	−1.833				
191	+2.118				
177	−0.023				
151	−0.129				

Table IV also contains calculated rotational strengths for apoaranotin (**3**), for dethioapoaranotin, and for a partially reduced dethioapoaranotin in which the cyclohexadiene ring has been half-reduced (**9**).

Discussion

The circular dichroism of gliotoxin appears from our calculations to have even greater complexity than is indicated by a superficial count of the number of CD bands. Moreover, in order to understand this complex curve it is necessary to take interchromophoric

interactions into account. Let us examine each of the longer wavelength bands in some detail.

(a) The 340-nm Band. This band can be safely assigned as the low-energy n → σ^* transition of the disulfide. The long-wavelength position of this band is a consequence of the small (*ca.* 15°) dihedral angle of the disulfide.[23,28,29] The negative sign is consistent with the left-handed chirality of the disulfide bond in gliotoxin.[12] However, the magnitude of the rotational strength is significantly smaller than one would expect. Both Linderberg and Michl's[29] and Woody's[23] theoretical treatments of the intrinsic disulfide rotational strength of disulfides predict a rotational strength of about −0.1 to −0.15 DBM for a dihedral angle of 15°. This is reduced to about −0.02 in gliotoxin. The theoretical calculations (Table III) predict this reduction, giving a value of −0.03 DBM. The principal source of this reduction is the coupling of the $n_1\sigma^*$ disulfide transition with the peptide $\pi\pi^*$ transitions in the DKP. This can be seen by comparing the curves in Figure 11 for diene + disulfide and diene + DKP with the disulfide curve in Figure 10. Interaction of the diene and disulfide leads to only a minor change in the amplitude of the 340-nm band, while the coupling of the DKP and disulfide groups causes about a fourfold reduction in the amplitude.

This strong effect of coupling on the long-wavelength disulfide band implies that even the sign of this band may be altered under certain conditions. Indeed, the CD spectrum of sporidesmin (Figure 4) has a positive band at *ca.* 360 nm, although the disulfide bond has left-handed chirality.[12] Similarly, chaetocin has a negative CD band in the region of 375 nm (Figure 5), although the disulfide group is known to have a right-handed chirality from X-ray diffraction work.[5]

These exceptions to the usual chirality rule for disulfides[30,31] are readily understood on the basis of our results. In sporidesmin[3b] and chaetocin,[5] the disulfide dihedral angle is appreciably smaller (*ca.* 10 and 8°, respectively) than it is in gliotoxin[13] (*ca.* 15°) and aranotin[16] (15–18°). Therefore the intrinsic rotational strength of the disulfide $n_1\sigma^*$ transition will be smaller[23,29] and more readily reversed in sign by coupling with the peptide groups. In addition, the indolinyl chromophore common to these antibiotics may contribute to the sign reversal through a coupled oscillator interaction. It is clear that *one cannot depend upon the sign of the long-wavelength band in epidithiapiperazinediones as an indicator of disulfide chirality.*

The solvent effects observed on the 340-nm band of gliotoxin (Table II) are not easily interpretable. There is only a 2-nm difference in the position of the band among the four solvents studied, with no clear-cut trend according to polarity; *e.g.*, the maximum is at 337 nm in both methanol and dioxane. The intensity is essentially constant for three of the four solvents but is anomalously low in trifluoroethanol, for which we have no explanation at present.

(b) The 270-nm Diene Band of Gliotoxin. The positive 270-nm CD band of gliotoxin was assigned by Beecham and Mathieson[10] as the long-wavelength $\pi\pi^*$ transition of a conjugated hexadiene. Beecham and Mathieson attributed the failure of the skewed diene rule[11] in this case to perturbation of the nodal structure of the diene π electrons by substituents allylic to double bonds. Beecham and coworkers[32,33] have developed this idea further and enunciated an "allylic oxygen rule" which states that if an oxygen is allylic to a diene and the chirality of the system C=CCO is right handed, there will be a positive contribution to the long-wavelength diene band, while if the system is left handed, there will be a negative contribution. Beecham and coworkers have noted that most of the apparent violations of the skewed diene rule (including gliotoxin) have allylic oxygen with a C=CCO chirality opposite to that of the diene and suggest that the allylic oxygen effect may outweigh the diene contribution in many cases where they are opposed to one another. However, Beecham and coworkers have not given any detailed discussion of how this allylic oxygen effect arises.

Ziffer, *et al.*,[14] concluded that the apparent failure of the skewed diene rule in gliotoxin results from an interaction of the disulfide and the diene. They suggested that coupling of the 270-nm diene band and a disulfide $n\sigma^*$ transition, which they assumed to lie at *ca.* 280 nm, might shift the diene band to sufficiently high energies that it could be associated with the strong negative CD band at 233 nm, consistent with the left-handed diene chirality. There are several difficulties with this argument, however. First, as noted by Beecham, *et al.*,[32] there is no reason to expect a disulfide transition in the 280-nm region for a disulfide with a dihedral angle of *ca.* 10°. Second, it is difficult to envision a coupling mechanism sufficiently strong to shift the diene band by nearly 1 eV from 270 to 233 nm. Third, if the diene band were shifted by this large amount to *higher* energies, general quantum mechanical considerations would require a similar shift of the putative disulfide 280-nm transition to *lower* energies and thus leave the 270-nm band unaccounted for. Finally, the absorption spectrum of gliotoxin[15] shows a maximum at *ca.* 270 nm with $\epsilon_{max} \sim 5000$, a value which is quite reasonable for the diene transition but much too large for any disulfide $n\sigma^*$ transition.

There is little doubt that the diene contributes to the 270-nm band in gliotoxin. As we shall see later, there is good reason to believe that another band contributes also in this region. Our calculations indicate that the diene CD is strongly affected by coupling with the disulfide group and to a lesser extent with the DKP group. The rotational strength of the 270-nm band of the diene is reduced from −0.38 DBM for the isolated diene to −0.24 DBM for diene + DKP. For the diene + disulfide, $R = -0.15$ DBM. Finally, when all three chromophores are considered together, $R = +0.076$ DBM. These effects can be clearly seen by comparing the appropriate CD curves in the 270-nm region of Figures 10 and 11. The disulfide transition which couples most strongly with the diene is the $n_3\sigma^*$ transition[23] which we have located at 233 nm. This transition has a large magnetic dipole transition moment (0.7 BM) directed perpendicular to the mean di-

(28) G. Bergson, *Ark. Kemi*, **12**, 233 (1958).
(29) J. Linderberg and J. Michl, *J. Amer. Chem. Soc.*, **92**, 2619 (1970).
(30) M. Carmack and L. A. Neubert, *ibid.*, **89**, 7134 (1967).
(31) R. M. Dodson and V. C. Nelson, *J. Org. Chem.*, **33**, 3966 (1968).
(32) A. F. Beecham, A. Mc L. Mathieson, S. R. Johns, J. A. Lamberton, A. A. Sioumis, T. J. Batterham, and I. G. Young, *Tetrahedron*, **27**, 3725 (1971).
(33) A. F. Beecham, *ibid.*, **27**, 5207 (1971).

sulfide plane. In gliotoxin, this magnetic moment is nearly parallel to the large electric dipole transition moment of the first $\pi\pi^*$ diene transition, which is along the long axis of the diene. Thus the geometry is ideal for a large $\mu \cdot m$ contribution[34] to the diene rotational strength.

We note within the framework of our calculation, the allylic oxygen effect proposed by Beecham and coworkers does not manifest itself. This is evident from the large negative rotational strength at 270 nm calculated for the diene alone (Figure 10). The allylic oxygen enters into this calculation through the contribution which its dipole field makes to the mixing of the diene $\pi-\pi^*$ transitions (the one-electron mechanism[35,36]). However, in the skewed diene, the one-electron mechanism only mixes the first $\pi\pi^*$ transition of the diene with the second transition and the latter is electrically and magnetically forbidden in the π-electron approximation which we have used here. Therefore one would only expect an appreciable effect of the allylic oxygen in our theoretical picture if one extended the treatment of the diene to take $\sigma-\pi^*$ and $\pi-\sigma^*$ excitations into account.

We conclude that the origin of the anomalous sign of the 270-nm band in gliotoxin is in part due to coupling of the diene and disulfide transitions. In addition, although we cannot exclude some contribution from an allylic oxygen effect, there is a further dominating factor discussed in the next section. Our interpretation is consistent with the experimental results of Ziffer, *et al.*,[14] on dethiogliotoxin and supports their basic premise. However, the diene–disulfide interaction manifests itself in an actual sign reversal of the 270-nm diene band and not in the large energy shifts which they proposed.

(c) The 270-nm Band of Aranotin and the 310-nm Bands of Gliotoxin and Aranotin. Consideration of the CD curve of acetylaranotin (Figure 3) strongly suggests that the diene is not solely responsible for the 270-nm band of gliotoxin. In acetylaranotin we find a positive CD band at 268 nm with a rotational strength comparable in magnitude to the 270-nm band of gliotoxin (Table I). This band cannot be ascribed to the divinyl ether chromophores which replace the diene of gliotoxin. Methyl vinyl ether itself has its first absorption maximum at 190 nm.[37] Substitution, ring effects, and coupling of the two vinyl ether groups apparently shifts this band to *ca.* 230 nm in aranotin, for the absorption spectrum[15] exhibits a rather intense shoulder in this region which can only be ascribed to the divinyl ether groups.

What is the source of the 268-nm CD band of aranotin? Nagarajan, *et al.*,[15] attributed this band and the 310-nm CD shoulder to the disulfide group. However, there is no evidence from absorption spectra,[30,31] CD spectra,[30,31,38] or theoretical calculations[23,24] on simple disulfides with dihedral angles of *ca.* 30 and 60° to suggest that the disulfide group should have absorption bands in the 270- or 310-nm regions for dihedral angles of 10–20°. We can also exclude the peptide group as a locus for electronic transitions in this low-energy region.

Having excluded all of the individual chromophores, we are led to the conclusion that the 270- and 310-nm bands of aranotin arise from a composite chromophore, presumably a charge-transfer transition. Charge-transfer transitions have been clearly demonstrated by Leonard and coworkers[39,40] in 1-thiacyclooctan-5-one, where a S → CO charge-transfer interaction occurs across the ring and manifests itself in a new absorption band with a maximum between 226 and 242 nm, depending on solvent polarity. Earlier, Fehnel and Carmack[41] observed that β-keto sulfides have rather strong bands (ϵ_{max} ~300) at about 300 nm, while β-carbalkoxy sulfides have a band of comparable intensity near 240 nm. However, solvent shift studies of Bergson and Delin[42] have shown that the 300-nm band of β-keto sulfides is blue shifted in solvents of increasing polarity, in contrast to the case with the cyclic keto sulfide studied by Leonard, *et al.*[39,40] Bergson and Delin therefore concluded that the 300-nm band of β-keto sulfides does not correspond to the same type of charge-transfer transition as the band observed by Leonard, *et al.* It may be simply a carbonyl $n\pi^*$ transition enhanced in intensity through perturbation by the nearby sulfur. On the other hand, the 250-nm band observed in molecules with ester groups β to a sulfur[41] may represent a charge-transfer transition.

One additional piece of circumstantial evidence favoring charge-transfer interactions between the disulfide and the peptide groups of the DKP ring is the fact that in all of the crystal structures determined thus far for these systems,[3b,5,13,16] the disulfide bridge is skewed in such a way that the sulfurs are closer to the carbonyl carbons than to the nitrogens by 0.1–0.2 Å. This indicates a special interaction between the sulfurs and the carbonyl carbons, since the nonbonded radius of carbon is larger than that of nitrogen by *ca.* 0.1 Å.[43]

We therefore carried out calculations of the rotational strength to be expected for transitions from the MO's made up from disulfide lone-pair orbitals into the antibonding π^* MO's of the peptide groups. These calculations, described in detail in the Appendix, have yielded results in rather good accord with experiment, considering the difficulty of this type of calculation. In particular, the transition from the highest filled disulfide orbital, n_1, which one would expect to give rise to the lowest energy CT transition, is predicted to have a negative rotational strength for the known absolute configuration of gliotoxin and aranotin. The magnitude is 6–7 times larger than that observed for the 310-nm band. In addition, our picture of the disulfide energy levels[23,24] would suggest that the CT bands $n_2\pi^*$ and $n_3\pi^*$ should be approximately degenerate. The calculated rotational strength for these two bands taken together is positive and within a factor of 4.5 of the observed 270-nm band of aranotin (Table III).

The same bands are expected in gliotoxin and in all other epidithiapiperazinediones with minor variations

(34) J. A. Schellman, *Accounts Chem. Res.*, **1**, 144 (1968).
(35) E. U. Condon, W. Altar, and H. Eyring, *J. Chem. Phys.*, **5**, 753 (1937).
(36) I. Tinoco, Jr., *Advan. Chem. Phys.*, **4**, 113 (1962).
(37) Reference 26, p 27.
(38) A. F. Beecham, J. W. Loder, and G. B. Russell, *Tetrahedron Lett.*, 1785 (1968).
(39) N. J. Leonard, T. L. Brown, and T. W. Milligan, *J. Amer. Chem. Soc.*, **81**, 504 (1959).
(40) N. J. Leonard, T. W. Milligan, and T. L. Brown, *ibid.*, **82**, 4075 (1960).
(41) E. A. Fehnel and M. Carmack, *ibid.*, **71**, 84 (1949).
(42) G. Bergson and A.-L. Delin, *Ark. Kemi*, **18**, 489 (1962).
(43) L. Pauling, "The Nature of the Chemical Bond," 3rd ed, Cornell University Press, Ithaca, N. Y., 1960, pp 224 and 263.

in position. The 270-nm band should be relatively less sensitive to dihedral angle than the 310-nm band. The latter should be red shifted with decreasing disulfide dihedral angle. More importantly, we would expect the signs of these bands to reflect the absolute configuration of the disulfide nucleus and hence each band should have the same sign in aranotin, gliotoxin, and sporidesmin and the opposite sign in chaetocin.

Our hypothesis of CT transitions is perfectly consistent with the results on gliotoxin, accounting for the observed 310-nm band and providing additional positive rotational strength in the 270-nm region. In fact, a comparison of the rotational strengths of the 270-nm bands in gliotoxin and aranotin suggests that the band in gliotoxin is dominated by the charge-transfer contribution and that the diene makes only a small contribution. If one assumes that the CT contributions are identical for these two systems, the diene must contribute a small negative rotational strength in gliotoxin. However, although we have assumed that the geometry of the epidithiapiperazinedione nucleus is identical for gliotoxin and aranotin, there is evidence[16] that there is not an exact identity and therefore the charge-transfer contribution may differ. In any event, the diene contribution to the 270-nm CD band of gliotoxin must be small, whether it is positive or negative.

In sporidesmin (Figure 4), there is no evidence in the observed CD curve for the expected negative 310-nm band, but it may be obscured by the much stronger positive band at 300 nm due to the indolinyl chromophore. There is a 270-nm positive band, comparable in strength to that of aranotin which is not ascribable to the indolinyl chromophore. In chaetocin (Figure 5), we find a weak *positive* shoulder at *ca.* 345 nm and a *negative* band at 270 nm of about the same strength as that of aranotin. This reversal in sign is consistent with our expectations, as is the shift of the first CT band to longer wavelengths.

The solvent shift data on gliotoxin (Table II) are not of much value in our assignment of the 310- or 270-nm bands. As with the longest wavelength band, the shifts are very small and unsystematic. Again, the most dramatic solvent effect is on the intensity of the 310-nm band in trifluoroethanol, for which we have no ready explanation.

For acetylaranotin, however, the solvent shifts in the 270-nm band, though small, show a systematic red shift with more polar solvents. This is consistent with an excited state which is more polar than the ground state and is characteristic of charge-transfer transitions. Presumably the lack of a clear trend for this band in gliotoxin is due to differing solvent dependencies of the diene $\pi\pi^*$ transition and the charge-transfer band.

(d) The 233-nm Band of Gliotoxin. Beecham and Mathieson[10] assigned the very intense 233-nm band of gliotoxin to peptide $n\pi^*$ transitions in the DKP ring. These transitions are expected to occur in this region,[21] but according to our calculations (Table III), the $n\pi^*$ transitions account for only a part of the total CD. Theoretical considerations on simple disulfides[23,24] suggest that two disulfide $n\sigma^*$ transitions should also lie in this region for small dihedral angles and these are quantitatively more important than the peptide $n\pi^*$ transition. We might note that the rotational strength of the $n_3\sigma^*$ transition would be expected to be *positive* for a left-handed disulfide group.[23] This transition, however, is the one which couples strongly with the 270-nm diene band, and this coupling makes a large *negative* contribution to the rotational strength of the $n_3\sigma^*$ transition. The total calculated rotational strength for the 233-nm band is about half as large as the observed value.

The 233-nm band shows a clear solvent dependence in gliotoxin. There is a total blue shift of 5 nm on going from dioxane to trifluoroethanol with the solvents being ordered according to polarity. This trend is clearly consistent with the assignment of this band to transitions which arise from nonbonding electrons on the sulfurs and the carbonyl oxygens. There are no significant changes in intensity with solvent.

(e) The 230-nm Bands of Aranotin and Chaetocin. In aranotin we expect that the peptide $n\pi^*$ transitions and the $n_2\sigma^*$ and $n_3\sigma^*$ transitions of the disulfide will occur in the 230-nm region, as discussed in (d) for gliotoxin. However, we have an additional contribution in this region for aranotin arising from the divinyl ether chromophores. In a Dreiding model, the double bonds of each divinyl ether group have a left-handed chirality (Figure 9) and so we may expect the coupled-oscillator interaction between them to lead to a negative long-wavelength transition.[34] From the absorption spectrum,[15] we infer that this band is centered at 229 nm. Our calculations on both aranotin (Table III) and on the isolated divinyl ether groups show that the divinyl ether chromophores do indeed make large negative contributions in the 230-nm region. This is the principal factor which makes the 230-nm band of aranotin about twice as strong as the corresponding band in gliotoxin.

In chaetocin,[5a] there is a strong positive CD band at 238 nm. Hauser, *et al.*,[5a] have suggested that this band has the same origin as the 233-nm band of gliotoxin. In verticillin,[44] which is closely related to chaetocin, there is a positive CD band at 236 nm. Minato, *et al.*,[44] have suggested that the opposite sign of this band *vis-à-vis* the 233-nm bands of gliotoxin, aranotin, and sporidesmin provides evidence of an opposite absolute configuration.

We would urge caution in applying the sign of the 233-nm band as a criterion of the absolute configuration of the epidithiapiperazinedione nucleus in cases where other chromophores contribute. Chaetocin shows a distinct shoulder in the absorption spectrum at *ca.* 240 nm (Figure 5) which is attributable to the indolinyl chromophore. This chromophore also undoubtedly contributes to the 238- and 236-nm bands of chaetocin and verticillin, respectively. In these two cases, as well as in aranotin, the additional chromophores absorbing in the 230–240-nm range happen to reinforce the contribution of the epidithiapiperazinedione nucleus, but this need not always be the case.

(f) Higher Energy Transitions. At higher energies we expect a fourth disulfide $n\sigma^*$ transition at *ca.* 205 nm and bands arising from the amide $\pi\pi^*$ and diene and divinyl ether $\pi\pi^*$ transitions. It can be seen from Figures 2 and 3 that the theory is not adequate at shorter wavelengths. For gliotoxin there is clearly a missing negative contribution in the 200-nm region. This may well be due to the diene group. Weiss, *et*

(44) H. Minato, M. Matsumoto, and T. Katayama, *Chem. Commun.*, 44 (1971).

al.,[45] reported a second transition in steroidal cyclohexadienes at *ca.* 205 nm which they assumed to be a second $\pi\pi^*$ transition. Our PPP calculations on the diene place the second $\pi\pi^*$ transition at 191 nm (after introducing the shift of energy of -0.7 eV mentioned above), but this is a forbidden transition and thus is not in accord with the rather intense transition observed by Weiss, *et al.* The third $\pi\pi^*$ transition is located at 177 nm according to our calculations. Thus, our calculations may have over estimated the energy of the higher $\pi\pi^*$ transitions or there may be a $\pi\sigma^*$ or $\sigma\pi^*$ transition in the 200-nm region. Further work needs to be done on this higher energy region of dienes. In aranotin, the discrepancy between theory and experiment in the 200-nm region is not as great as in gliotoxin, but there is no doubt room for improvement of our understanding in this system as well.

Let us now briefly consider the CD spectra of various derivatives of aranotin and of apoaranotin. The CD spectrum of dethiodeacetylaranotin (**6**), shown in Figure 6, has a negative band near 222 nm comparable with that in aranotin which is attributable to the peptide $n\pi^*$ and the divinyl ether $\pi\pi^*$ transitions. There is a second negative band near 200 nm with about half the intensity of the 222-nm band. Our calculations are consistent with these results (Table IV), yielding negative bands at about 230 and at 200 nm.

On reducing one double bond in one of the rings to form **7**, the CD spectrum shows about half the intensity in the 222-nm band and an increase in intensity of the 200-nm band. This can be interpreted as reflecting the loss of one divinyl ether chromophore contribution to the long-wavelength band and the gain of a vinyl ether chromophore at about 200 nm. The theoretical results support this: the long-wavelength bands for **7** are weaker than for **6**, and a strong negative band appears at 216 nm, associated with the vinyl ether moiety.

In **8**, with both seven-membered rings half-reduced, the long-wavelength band becomes positive while the 200-nm band increases still further. The theoretical results yield a very weak negative band at long wavelengths, reproducing the observed trend but not carrying it sufficiently far. This may reflect a real inadequacy in the theory, or may be attributable to changes in the conformation of the system upon reduction. Again, the vinyl ether groups are predicted to make a large negative contribution, although the calculated position of the band is red shifted relative to experiment.

For acetylapoaranotin (**3b**), the observed CD spectrum (Figure 7) is very much like that of gliotoxin (Figure 2) except that the 230-nm band is considerably stronger and there is a shoulder at about 200 nm. Our calculations (Table IV) are consistent with these results in the case of the two long-wavelength bands. However, for apoaranotin, we find that the diene band at 270 nm is still weakly negative and we do not reproduce the observed positive CD band at 270 nm. The calculations do predict a 230-nm band intermediate in magnitude between that of gliotoxin and aranotin, in agreement with experiment. As with the latter two systems, there is very poor agreement in the higher energy region below 210 nm.

(45) U. Weiss, H. Ziffer, and E. Charney, *Tetrahedron*, **21**, 3105 (1965).

For dethioapoaranotin, we predict (Table IV) a strong negative band at 270 nm due to the diene and a negative band near 230 nm which is stronger than that of gliotoxin. This derivative has not yet been studied experimentally due to the difficulty of cleanly removing the disulfide without reducing any double bonds.

In dihydrodethiodeacetylapoaranotin (**9**), we observe (Figure 7) a negative band at 220 nm and a stronger negative band at 200 nm. Theory (Table IV) predicts a negative band due to the DKP $n\pi^*$ and divinyl ether $\pi\pi^*$ transitions at *ca.* 230 nm and a stronger negative band at *ca.* 200 nm from the DKP, divinyl ether, and vinyl ether $\pi\pi^*$ transitions.

In Figure 8, we see that in the fully reduced dethio derivative of aranotin (**10**) the CD spectrum shows a negative band near 222 nm and a positive band at 210 nm. This spectrum is very similar to that of L-prolyl-L-proline diketopiperazine (**11**), except for a wavelength shift of several nanometers. Since the only remaining chromophore in this system is the diketopiperazine ring, this can be compared with the calculations for that isolated chromophore given in Figure 10. It can be seen that the agreement is qualitatively very good; the $n\pi^*$ transition is negative and the long-wavelength component of the $\pi\pi^*$ transition is positive.

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Appendix

Calculation of Rotational Strengths for Charge-Transfer Transitions. The rotational strength of an intramolecular charge-transfer transition can be calculated from the expression[19]

$$R = \frac{-e^2\hbar^3}{2m^2cE}(\psi_0|\nabla|\psi_{CT})\cdot(\psi_{CT}|\mathbf{r}\times\nabla|\psi_0) \quad \text{(A-1)}$$

where e, $\hbar$, m, and c are, respectively, the electronic charge, Planck's constant divided by 2π, the mass of the electron, and the velocity of light. E is the energy of the transition from the ground state (ψ_0) to the excited state (ψ_{CT}). For R in Debye Bohr magnetons, E in electron volts, and the ∇ operator in Å^{-1} ($\mathbf{r}\times\nabla$ being dimensionless)

$$R = \frac{36.5964}{E}(\psi_0|\nabla|\psi_{CT})\cdot(\psi_0|\mathbf{r}\times\nabla|\psi_{CT}) \quad \text{(A-2)}$$

Representing ψ_{CT} as a determinantal wave function

$$\psi_{CT} = \psi_D{}^A = \frac{1}{\sqrt{2}}[|\Phi_D{}^\alpha\Phi_A{}^\beta| - |\Phi_D{}^\beta\Phi_A{}^\alpha|] \quad \text{(A-3)}$$

where D is the donor MO and A is the acceptor MO, and the α and β represent spin wave functions.

Since our MO's are localized on different groups in the molecule (donor orbitals in the disulfide and acceptor orbitals in the amide), we must evaluate the appropriate two-center integrals. The distances between centers are substantially greater than bonding distances ($\gtrsim$2.65 Å) and single-exponential Slater orbitals, due to their rapid decay beyond bonding distances,[46] will lead to underestimates of these two-center

(46) R. S. Mulliken, C. A. Rieke, D. Orloff, and H. Orloff, *J. Chem. Phys.*, **17**, 1248 (1949).

integrals. Therefore we have used atomic SCF orbitals given by Clementi,[47] which are expressed as linear combinations of four to eight Slater functions of the proper symmetry.

The atomic orbitals involved make arbitrary angles with respect to the internuclear vectors. Therefore a computer program LOCAL written by A. E. Hansen[48] was used to express each orbital in terms of a σ component directed along the internuclear axis and two components (π and $\bar{\pi}$) perpendicular to the internuclear axis. The dipole velocity matrix elements for various symmetry types ($\sigma\sigma$, $\sigma\pi$, $\pi\pi$, etc.) were calculated using expressions derived by Imamura *et al.*,[49] by Král,[50] and by Woody.[23,51] These were then combined with the MO coefficients to yield the necessary dipole velocity matrix elements.

The angular momentum matrix elements were calculated using a similar approach. These reduce[49,52] to terms involving $\mathbf{R}_i \times \boldsymbol{\nabla}_{ji}$ (where $\mathbf{R}_i$ is the vector from the origin (the midpoint of the disulfide bond) to atom i, and $\boldsymbol{\nabla}_{ji}$ is the dipole velocity matrix element between orbitals centered on atoms j and i) and terms involving overlap integrals.

The dipole velocity and angular momentum matrix elements obtained in this way are small but not negligible. However, one must take into account the mixing of the charge-transfer states with locally excited states in the amide and the disulfide. Although this mixing is relatively small, it makes a significant contribution to the charge-transfer matrix elements because the amide $\pi\pi^*$ and $n\pi^*$ have large $\boldsymbol{\nabla}$ and $\mathbf{r} \times \boldsymbol{\nabla}$ matrix elements, respectively, connecting them to the ground state. We calculate the Hamiltonian matrix element between the charge-transfer state and locally excited configurations as follows[53]

$$(\psi_A{}^D|H|\psi_{D'}{}^D) = -\beta_{AD'} = -kS_{AD'}$$

$$(\psi_A{}^D|H|\psi_{A'}{}^A) = \beta_{DA'} = kS_{DA'}$$

where $\beta_{AD'}$ and $\beta_{DA'}$ are matrix elements of the core Hamiltonian between the orbitals A and D′ and D and A′, respectively. We assume that these are proportional to the corresponding overlap integrals, with a proportionality factor, $k \cong -10$ eV. The overlap integrals were evaluated using the atomic SCF wave functions of Clementi,[47] as described above.

It was found that the matrix elements connecting the charge-transfer transitions with the disulfide $n\sigma^*$ transitions were sufficiently small that this type of mixing is negligible. However, the mixing with the peptide $n\pi^*$ and $\pi\pi^*$ transitions was not negligible. The matrix describing the mixing of these transitions was set up and diagonalized. From the mixing coefficients, the total $\boldsymbol{\nabla}$ and $\mathbf{r} \times \boldsymbol{\nabla}$ matrix elements for the charge-transfer transitions were calculated and from these the rotational strengths follow, given the transition energies. The lowest energy CT transition, from the highest energy disulfide orbital n_1 to the amide π^* orbitals, was assumed to correspond to the 310–320-nm band observed in diketopiperazine disulfides. The next CT transitions, $n_2 \rightarrow \pi^*$ and $n_3 \rightarrow \pi^*$, were assumed to be degenerate and located at 270 nm. Their degeneracy is suggested by the calculations of Yamabe, *et al.*,[24] and is consistent with our treatment[23] of the $n_2 \rightarrow \sigma^*$ and $n_3 \rightarrow \sigma^*$ transitions localized in the disulfide group. Finally, the $n_4 \rightarrow \pi^*$ transition was rather arbitrarily placed at 235 nm.

In principle the energies of these charge-transfer bands should be calculable from theory. However, it is doubtful whether the presently accessible level of approximation is adequate for any accurate predictions. That such charge-transfer transitions are to be expected in the observed energy region can be seen from a rough calculation using the expression[11]

$$E_{CT} = I_D - A_A - Q$$

where I_D is the ionization potential of the donor orbital, A_A is the electron affinity of the acceptor orbital, and Q is the Coulomb integral between the "hole" in the donor orbital and the electron in the acceptor orbital.

I_D can be estimated for the n_1 orbital of the disulfide as follows. Bock and Wagner[54] report an ionization potential of 8.8 eV for simple open-chain disulfides. From the wavelength of the $n_1 \rightarrow \sigma^*$ transition in gliotoxin[15] (340 nm) and that of the $n_1 \rightarrow \sigma^*$ transition in open-chain disulfides[55] (~250 nm), we can estimate that the n_1 orbital in gliotoxin should have an ionization potential of about $8.8 - 1.3 = 7.5$ eV. The electron affinity of the π^* orbital in amides can be estimated from CNDO/S calculations on formamide[51] to be *ca.* -1.4 eV. Finally, approximate calculations[51] of Coulomb integrals using the Pariser–Parr[56] approach lead to an estimate of $Q \cong 4.1$ eV. This gives $E_{CT} \cong 7.5 + 1.4 - 4.1 = 4.8$ eV, corresponding to a wavelength of ~260 nm. Given the approximations involved in estimating the contributions to this charge-transfer energy, this rough calculation is quite consistent with the presence of such charge-transfer transitions in the 230–320-nm region.

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