and the solvent was evaporated under reduced pressure and the oily residue crystallized on standing. Four recrystallizations from benzene–hexane gave 0.1 g (30%) of a dihydro adduct as colorless needles: mp 157.5–158.5°; ir ($CHCl_3$) 1730 cm^{-1} (C=O); nmr ($CDCl_3$) δ 1.09 (d, 6 H), 2.05 (g, 2 H), 1.18 (s, 9 H), 1.62 (s, 3 H), 1.40 (t, 3 H, J = 7 Hz), and 4.36 (q, 2 H, J = 7 Hz); mass spectrum (70 eV) m/e 315.

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Disulfide Stereochemistry. Conformations and Chiroptical Properties of L-Cystine Derivatives[1]

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Abstract: Successive N-methylation of L-cystine (L-CySSCy (**1**)) reverses the relative magnitudes of the vicinal coupling constants and chemical shifts of the anisochronous methylene hydrogens on passing from di- to tetramethylcystine. These pmr results indicate that the most stable staggered ethanic rotamer with anti sulfur and carboxylate residues for **1** is succeeded by the rotamer with anti sulfur and methylated ammonium groups. For the same series of compounds, the composite circular dichroism (CD) of the disulfide absorption at >240 nm changes sign from negative to positive upon N-methylation, permitting estimation of contributions to optical activity by each rotamer due to perturbation of the disulfide chromophore by the asymmetric centers. Upon passing from water and methanol to longer chain alcoholic solvents, the disulfide CD of alkyl ester dihydrochlorides of **1** also changes sign from negative to positive, suggesting a similar change in rotamer preference. Acylation and amidation of **1** produce only minor effects on vicinal coupling constants and CD, indicating only small influences of these substituents on rotamer distribution. Mixed disulfides such as L-$CySSC_2H_5$ yield coupling constants and CD curves similar to that of **1**. These results among others indicate no significant restriction on the conformation of **1** due to endocyclic interactions. The relatively high optical rotatory properties of **1** and derivatives in solution are due not to endocyclic interactions nor to biasing of screw sense in the disulfide bond but rather to unequal populations of three staggered rotamers. From an examination of (−)-(9*S*,10*S*)-*trans*-hexahydro-2,3-benzodithiin and crystals of **1**, a negative long wavelength CD sign is associated with M disulfide chirality for dihedral angles less than 90°. The utility of the long wavelength CD in assigning M disulfide chirality and monitoring conformational changes is demonstrated for the naturally occurring cyclopentapeptide malformin A.

Two decades have passed since a rekindling of interest in the high optical rotation of L-cystine (L-CySSCy (**1**)) relative to L-cysteine (L-CySH (**2**)) and other amino acids. A postulate[3] that endocyclic interactions composed of hydrogen bonds make **1** more akin to the cyclic amino acid L-proline than to the less strongly rotatory acyclic amino acids followed an earlier suggestion[4] of ". . . forces which very severely restrict the freedom or orientation of groups . . ." in **1**. When the proposal of endocyclic interactions appeared, it was quickly countered by the point that proximity of an asymmetric center to a disulfide bond is sufficient to produce increased D-line rotation.[5] As increasingly more sophisticated instrumentation has become available, optical rotatory dispersion[6-10] and circular dichroism[10-12] data have been reported for **1**.

The disulfide bond is potentially an inherently dissymmetric chromophore, for the normal disulfide dihedral angle is near 90°, and in the presence of a chiral center, some degree of preference for M (left-handed) or P (right-handed)[13] disulfide chirality must be induced.[6] In such an instance, two contributions considered additive to optical activity are present, that of the potentially chiral chromophore and that of the chromophore perturbed by the asymmetric center. In L-cystine, the last contribution may in turn be considered the sum of weighted contributions according to the relative

(1) Abstracted from the Doctoral Dissertation of Jeremiah P. Casey, University of Virginia, 1968. The research was supported by grants from the National Science Foundation.

(2) NDEA Fellow, 1964–1967; Tennessee Eastman Fellow, 1967–1968.

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Table I. Proton Magnetic Resonance Parameters of L-Cysteine and Derivatives at 100 MHz

Compound	Ion form	$-J_{AB}$	J_{AC}	J_{BC}	$\Delta\nu_{A-B}$	$\Delta\nu_{C-A/B}$[a]	$\Delta\nu_{A/B-Y}$[a]
L-CySH (**2**)	+		N = 4.85			124.3	
L-CySSCy (**1**)	+, +	15.3	4.4	7.6	12.7	112.6	
	−, −	13.6	4.6	7.7	21.9	55.2	
L-CySMe (**3**)	+	14.9	4.7	7.3	9.1	120.2	98.0[b]
	±	14.8	4.1	7.9	9.0	88.9	88.1[b]
	−	13.5	5.0	7.2	9.8	64.4	66.3[b]
L-CySSEt (**4**)	+	15.0	4.4	7.6	9.6	118.3	
L-CySSCH$_2$CH$_2$OH (**5**)	+	15.1	4.3	7.8	12.6	116.9	
	±	15.0	4.0	8.4	21.7	86.7	
GSH (**6**)	+		N = 6.15			161.8	108.3[c]
GSMe (**7**)	+	14.1	4.9	9.0	15.1	*d*	97.9[c]
GSSEt (**8**)	+	14.3	4.5	9.4	21.9	*d*	93.2[c]
GSSG (**9**)	+, +	14.2	4.4	9.4	25.7	*d*	86.7[c]
	±, ±	14.2	4.5	9.4	29.9	*d*	79.3[c]
N,N'-DiGly-L-CySSCy (**10**)	+, +	14.3	3.7	9.1	23.4	*d*	70.5[c]
	±, ±	14.1	3.9	8.6	24.1	*d*	75.2[c]
L-CySSCy-bis-Gly (**11**)	+, +	15.1	5.2	8.0	18.6	113.1	74.1[c]
	±, ±	15.0	5.5	8.0	17.5	108.1	51.5[c,e]
N,N'-DiMe-L-CySSCy (**12**)	+, +	15.6	5.3	5.2	8.2	94.1	56.9[f]
	±, ±		N = 5.5			60.7	59.0[f]
	−, −		N = 6.3			29.3	68.0[f]
N,N,N',N'-TetraMe-L-CySSCy (**13**)	+, +		N = 5.5			93.1	51.1[f]
	±, ±		7.7[g]	3.3[g]	−3.9[g]	54.8	54.9[f]
N,N,N,N',N',N'-HexaMe-L-CySSCy (**14**)	+, +	13.4	11.9	3.4	−31.8	56.4	51.6[f]
	±, ±	13.1	11.9	3.4	−31.3	24.1	40.0[f]
4-Carboxythiazolidine (**15**)	+	12.3	7.2	5.9	6.0	134.3	27.0[h,i]
3-Methyl-4-carboxy-thiazolidine (**16**)	+	12.2	8.4	5.9	21.8	108.2	18.1[h,j]

[a] Chemical shift in hertz from $(\nu_A + \nu_B)/2$. [b] Y = $-SCH_3$. [c] Y = Gly-CH_2-. [d] Methine signal obscured by HDO lock interference. [e] $-CH_2COO^-$ a quartet; $\Delta\nu_{A-B}$ = 10.3 Hz, J_{AB} = 17.4 Hz. [f] Y = $N(CH_3)_n$. [g] Calculated, see text. [h] Y = SCH_2N. [i] For Y, J_{AB} = 10.2, $\Delta\nu_{A-B}$ = 8.2 Hz. [j] For Y, J_{AB} = 10.4, $\Delta\nu_{A-B}$ = 37.1 Hz.

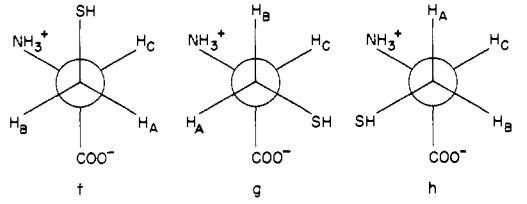

Figure 1. Three staggered rotamers of L-cysteine.

populations of three rotamers of a cysteine-like residue considered as a substituted ethane. Following an earlier study[14] of **2** and its derivatives, we have undertaken a conformational analysis by proton magnetic resonance of **1** and closely related species in order to separate the contributions of asymmetric perturbation from inherent dissymmetry as sources of optical activity in chiral disulfides.

Proton magnetic resonance (pmr) spectra of **2** and its derivatives exhibit from 5 to 12 lines due to the three-spin ABX or ABC type system.[14-16] These spectra are time-averaged over three predominant staggered rotamers, designated for the purpose of labeling and expressing mole fractions as *t*, *g*, and *h*, where the bulky carboxylate and sulfhydryl groups are anti or trans in the *t* rotamer, gauche in the *g* rotamer, and also gauche in the most hindered *h* rotamer, with the bulkiest groups of **2** in adjacent positions as shown in Figure 1. The labeling of H_A and H_B in Figure 1 involves a commitment to the designation of the H_B proton anti to H_C in rotamer *t* as that one of the AB pair which is at higher field in **2**. Arguments for this designation in **2** and derivatives have been presented.[14] When the pmr spectra exhibit a sufficient number of lines, spin coupling constants J_{AC} and J_{BC} may be individually determined and related to the vicinal coupling constants J_G and J_T for the gauche and anti rotamers

$$J_{AC} = tJ_G + gJ_T + hJ_G$$

$$J_{BC} = tJ_T + gJ_G + hJ_G$$

The difference

$$J_{BC} - J_{AC} = (t - g)(J_T - J_G) \quad (1)$$

and, since $J_T > J_G$, $J_{BC} > J_{AC}$ implies that rotamer *t* is favored over rotamer *g*. If values of J_T and J_G are assigned from model compounds, quantitative estimates of rotamer populations may be made.

Results

Proton Magnetic Resonance. Table I lists coupling constants and chemical-shift differences for derivatives of **2** in aqueous media. The sum of the vicinal coupling constants $2N = J_{AC} + J_{BC}$ is the same 12.0 Hz for **1, 3,** and **4** in acid solutions and is unaltered with a 96° change in temperature. This result indicates, according

$$2N = J_{AC} + J_{BC} = J_G(1 + h) + J_T(1 - h) \quad (2)$$

to the sum given in eq 2, a constant population of rotamer *h* and suggests a mole fraction near $^1/_3$. Over the same temperature range, the difference $J_{BC} - J_{AC}$ decreases, indicating the populations of rotamers *t* and *g* are becoming equal (eq 1).

A separate pH study of *S*-methyl-L-cysteine (L-CySMe (**3**)), wherein pmr spectra were recorded and analyzed for every 0.25 equiv of added base or acid to give anion or cation forms, established that the C,

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Table II. Alkyl Disulfide Pmr Parameters[a]

Compound	Solvent (ref)[b]	$-J_{AB}$	J_{AX}	J_{BX}	$\Delta\nu_{A-B}$
trans-2,3-Dithiadecalin (**17**)	CCl_4 (TMS)	13.4	10.3	1.6	22.8
	CCl_4 (C_6H_6)	13.4	10.2	1.8	31.7
Active amyl disulfide (**18**)	C_6H_6 (C_6H_6)	12.8	5.9	7.1	21.3

[a] In Hz at 100 MHz. [b] Internal reference.

A, and B resonances occur in that order going to higher field at all pH values and do not cross.[17] For **2** the absence of crossover cannot be established with certainty, due to the collapse of the ABX type spectrum to a deceptively simple ABX spectrum at low pH.

Acylation or amidation of **1** alters only slightly the vicinal coupling constants and hence the preference for the substituted ethanic rotamer with anti sulfur and carboxylate groups. Compounds **6–11** including glutathione, GSH, are all glycine derivatives of **2**. Among them only **11** as the neutral species in D_2O exhibits observable anisochrony of the glycyl methylene protons. This quartet was not observed in the 60-MHz pmr spectrum of a commercial sample of the peptide previously reported, but its observation only in the dipolar ion form is consistent with earlier interpretations of the anisochrony.[18]

Increasingly, N-methylated L-cystines **12–14** are soluble in both acidic and neutral water solvent. Monomethylation of each amino group in **1** to give **12** increases J_{AC} and decreases J_{BC} so that they are nearly equal, indicative of an evening of the populations of rotamers *g* and *t*. Though its spectrum consists of but seven lines in D_2O, assuming a value for J_{AB} permits calculation of vicinal coupling constants for tetramethyl-L-cystine (**13**) by simultaneous solution of equations for a deceptively simple ABX spectrum.[19] Since in the calculation both $J_{AX} - J_{BX}$ and $\Delta\nu_{A-B}$ are dependent upon $J_{AB}^{1/2}$, the assumed geminal coupling constant $J_{AB} = -14.5$ Hz is not critical for the final results of $J_{AX} + J_{BX} = 11.0$ Hz, $J_{AX} - J_{BX} = 4.4$ Hz, and $\Delta\nu_{A-B} = 3.9$ Hz. Hexamethyl-L-cystine (**14**) clearly differs from any of the other derivatives of **2** in that the upfield proton has the much higher vicinal coupling constant. Examination of space-filling models reveals that **14** is exceedingly crowded, and only a conformer near rotamer *g* is sterically possible. In terms of the designations made in Figure 1, the larger vicinal coupling constant is logically assigned as J_{AC}, requiring that the A rather than the B proton occurs at higher field. With the additional sign identification offered in Table I for **13**, a smooth variation of coupling constants and chemical shift parameters is obtained for successive N-methylation of **1** through **12**, **13**, and **14**. Thus, H_A appears at higher field than H_B in passing from **12** to **13** and the *g* rotamer is increasingly favored for successively methylated species. The changes upon N-methylation support the designations of A and B protons in Figure 1.

Table I concludes with a description of thiazolidine spectra recorded in properly identifying and synthesizing **13** following difficulties with a reported procedure.[20] The reported preparation of **13** *via* reductive formylation of **1** was reproduced with difficulty but the purported tetramethyl-L-cystine was identified by spectral methods as the free base of 3-methyl-4-carboxythiazolidine hydrochloride (**16**) (see Experimental Section). Both **16** and 4-carboxythiazolidine (**15**) were used to unequivocally synthesize **13**. Some aspects of the difficulty in interpreting conformations for five-membered ring systems capable of pseudorotation have been discussed.[21,22]

In order to assess the importance of disulfide screw sense, a rigid system containing exclusively one known sense was synthesized and compared with a flexible analog. Table II presents pertinent pmr parameters for *rac-trans*-hexahydro-2,3-benzodithiin (*trans*-2,3-dithiadecalin (**17**)) and bis(2-methylbutyl) disulfide (active amyl disulfide (**18**)). In each compound, the X part of the ABX spectrum is complicated by further coupling and/or obscured by other protons, depending on the solvent used, so the entire ABX analysis was performed in the eight-line AB part of the spectrum.[23]

The high value of J_{AX} and low value of J_{BX} indicate the trans bicyclic compound **17** exists predominately as the rigid, locked di-chair conformer.[24] From the coupling constants in Table II, the low-field proton H_A adjacent to the sulfur must be in an axial position and the high-field proton H_B must be in the equatorial position. Increasing the temperature has little effect on either J_{AX} or J_{BX}. An alicyclic analog of **17**, **18**, exhibits more nearly equal vicinal coupling constants that are largely unaffected by changes in solvent polarity (CH_3OH *vs.* C_6H_{12}) and temperature (0–154°).

Circular Dichroism. For **1**, differential molar absorptivity ($\Delta\epsilon$) values for the negative extremum at 252 nm are identical for 1.0 *N*, 5.0 *N*, and saturated HCl solutions (Table III, Figure 2). For the zwitterion, reproducible CD curves were obtained by dissolving **1** in water and by dissolving the lithium salt of **1** in water and neutralizing the solution to pH 5 with HCl. Due to decomposition of the disulfide in alkaline media, the CD spectrum in base is reproducible only with freshly prepared solutions. The results presented are average values for either the lithium salt of **1** dissolved in water or **1** dissolved in 0.1 *N* NaOH. These results agree well with those reported for **1** in acid or base by several investigators[10–12] and with one value for neutral solution,[10] but not with other values for neutral solution.[12] The absorption spectrum of **1** in 0.1 *N* HCl

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Table III. Circular Dichroism Spectra of L-Cystine and Derivatives

Compound	Solvent[a]	$\Delta\epsilon(\lambda)$[b]	λ_0[c]	$\Delta\epsilon$ (λ)[d]
L-CySSCy (**1**)	HCl	−0.55 (252)	240	+5.5 (220)
	H_2O	−0.70 (257)	236	+4.1 221
	NaOH	−0.23 (270)	257	+2.5 210
N,N'-Diformyl-L-CySSCy (**19**)	CH_3OH	−0.44 (257)	242	+5.3 (221)
N,N'-Diglycyl-L-CySSCy (**10**)	HCl	−0.40 (258)	239	+5.0 (220)
	H_2O	−0.39 (265)	243	+2.0 215
L-CySSCy-bis-Gly (**11**)	HCl	−0.28 (257)	243	+5.7 (221)
	H_2O	−0.26 (257)	242	+5.2 215
L-CySSCy-bis-Ala (**20**)	HCl	−0.29 (257)	243	+9.9 (219)
L-CySSCy-bis-Leu (**21**)	HCl	−0.21 (257)	243	+11.1 (218)
GSSG (9)	HCl	−0.30 (262)	241	+1.6 (224)
	H_2O	−0.35 (265)	237	+0.7 (227)
L-CySSCy-di-*t*-Bu-di-HCl (**24**)	H_2O	−0.39 (255)	243	+4.4 (221)
	i-PrOH	+0.44 255		+6.5 (219)
L-CySSCy-di-Me-di-HCl (**23**)	MeOH	−0.38 (249)	241	
	n-PrOH	+0.05 262		
N,N'-Di-Me-L-CySSCy (**12**)	HCl	+0.05 (280)	264	
		−0.15 (247)	242	+5.7 (215)
	H_2O	−0.27 (257)	237	+4.5 (205)
	NaOH	−0.03 (288)	276	
		+0.20 (250)		+5.0 215
N,N,N',N'-Tetra-Me-L-CySSCy (**13**)	HCl	+0.42 258		+6.2 (210)
	H_2O	+0.30 255		+7.1 (208)
	NaOH	+0.90 250		+6.2 215
N,N,N,N',N',N'-Hexa-Me-L-CySSCy (**14**)	HCl	+0.90 250		+4.0 210
	H_2O	+0.80 250		+12.0 210

[a] HCl and NaOH refer to 0.1 *M* aqueous solutions. [b] Maximum wavelength indicated by parentheses in nm, otherwise broad, ill-defined peaks in these disulfide bands. [c] Crossover point where $\Delta\epsilon = 0$. [d] Maximum indicated by parentheses in nm, otherwise shortest carbonyl wavelength measured.

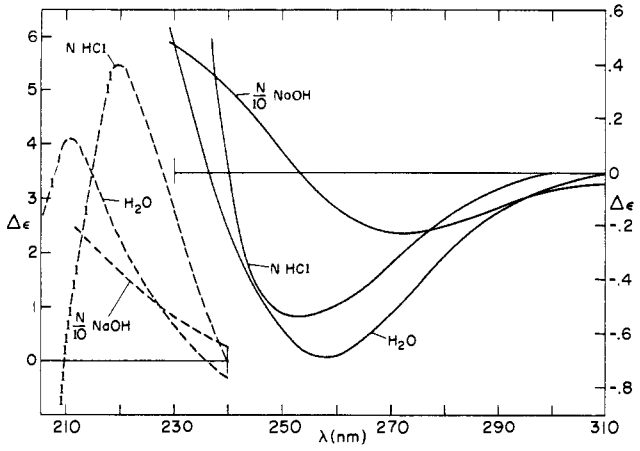

Figure 2. CD of L-cystine in aqueous solutions.

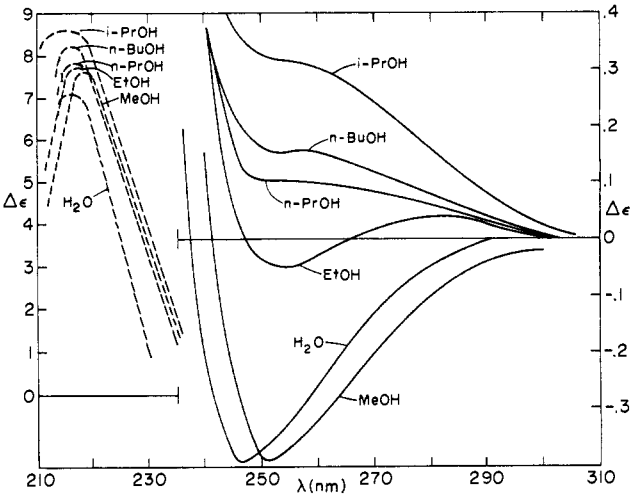

Figure 3. CD of L-cystine diethyl ester·2HCl in water and alcoholic solvents.

exhibits a broad shoulder at 244 nm (ϵ 320) and higher absorption at shorter wavelengths. In 0.1 *N* NaOH, a small distinct maximum is observed at 246 nm (ϵ 344), indicating the disulfide chromophore is not drastically altered by deprotonation of two carboxylic acid and two ammonium groups.

We concur with previous assignments[10] in considering the positive Cotton effect at *ca.* 220 nm for **1** to have its origin in the carboxyl group n–π^* transition. As found for other amino acids,[11,25] ionization of the carboxylic acid group shifts the observed CD to shorter wavelength and decreases $\Delta\epsilon$ (Figure 2). The longer wavelength of this carboxyl Cotton effect relative to that of other amino acids may result from vicinal carboxyl–sulfur group electronic interactions[26] which lower the carboxyl transition energy, or possibly from the presence of an intense negative CD at shorter wavelength. Such a high energy CD has been reported for **1**,[11] and, by overlap and mutual cancellation,[27] may suffice to explain the apparent longer wavelength of the carboxyl CD band.

Acylation or amidation of **1** diminishes the magnitude of the disulfide CD, but the band contours for **10**, **11**, and **19**–**21** (Table III) resemble that of **1** in neutral solutions. If no new asymmetric center is introduced, transitions from the newly formed amide groups result in no significant change from **1** for the carboxyl region CD of **10**, **11**, and **19**. Introduction of a new asymmetric center augments only the carboxyl region CD in

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(26) Short wavelength, more electric dipole allowed sulfur transitions presumably couple most strongly with the proximate carboxyl chromophore. Long wavelength sulfur transitions also reflect such vicinal perturbations; E. A. Fehnel and M. Carmack, *ibid.*, 71, 84 (1949).

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20 and **21**. Oxidized glutathione (**9**) also undergoes little change in disulfide region CD compared to **1**, but the carboxyl region optical activity is reduced for this compound. The CD result for **9** is in excellent agreement with a previous report.[10]

The qualitative similarity of the CD spectra of the many derivatives of **1** mentioned above does not, however, persist throughout *all* derivatives of **1**. Figure 3 presents a striking set of curves recorded for L-cystine diethyl ester dihydrochloride (**22**) in water and a series of homologous alcohols. We were led to this solvent effect study by the curious early observation that the dimethyl and diethyl ester dihydrochlorides of **1** exhibit oppositely signed rotations at the D line for water and methanol (−) *vs.* ethanol (+).[28] This observation was confirmed and the study extended to view the effects of still more nonpolar alcohols by CD. Despite the dramatic change in the observed CD in the disulfide region, from negative to strongly positive as solvent chain length increases, the carboxyl region CD is relatively unchanged. Uv spectra in each solvent exhibit a weakly defined disulfide absorption near 250 nm, with ϵ varying from 400 to 300 as the series progresses from water to isopropyl alcohol. The dimethyl and di-*tert*-butyl ester dihydrochlorides of **1** (**23** and **24**) also exhibit this sign crossover in the disulfide region without major changes in the carboxyl group region (Table III).

Certain comments on obtaining these results are pertinent. Well-dried alcoholic solvents are necessary for reproducible results. Transesterification does not readily occur and therefore cannot be the cause of the observed sign change. The possibility of transesterification was examined by attempting a representative exchange reaction. After a saturated solution of **22** in isopropyl alcohol was heated 0.5 hr and allowed to stand 2.5 hr, **22** was recovered quantitatively by precipitation with ether and was unchanged (mixture melting point). Concentration effects are unimportant, for varying solute concentration by a factor of 10 had no effect on the spectrum of **22** in absolute EtOH, nor does gegenion association appear to be the cause of the phenomenon, for saturation with KCl or HCl gas did not have any effect on the observed spectrum of **22** in absolute EtOH.

Circular dichroism spectra of the increasingly N-methylated cystines **12–14** also exhibit many qualitative changes depending on the degree of methylation (Figure 4) and acidity of the aqueous solvent (Table III). Among these three compounds, only the D-line rotations of **12** have been described.[29] As stated above, **13** has never been properly characterized, for that compound with $[\alpha]D$ −146°[20] in water is actually 3-methyl-4-carboxythiazolidine. The cystine betaine **14** had been previously synthesized,[30] but its optical rotation has never been reported.

Certain trends in the CD spectra of **1** and **12–14** are apparent. Increasing methylation gives a more positive CD in the disulfide long wavelength region without a sign change in the carboxyl region (Figure 4). In neutral solution the CD curve at 240–250 nm is more negative than in acid solution for all three compounds, reminiscent of the behavior of **1** (Figure 2). **14** decomposes rapidly in base, but **12** and **13** are sufficiently stable in 0.1 *N* NaOH to give reproducible spectra. As with **1**, deprotonation of the ammonium group results in more positive CD in the disulfide absorption region (Table III) relative to the zwitterion form. Upon increasing the temperature, both disulfide CD peaks of **12** in HCl decrease proportionately. The ultraviolet spectra of **12–14** in acid and water strongly resemble that of **1**. All show a broad, slightly defined disulfide absorption maximum at 246–250 nm in water (ϵ ~350) on curves showing greater absorbance at shorter wavelengths.

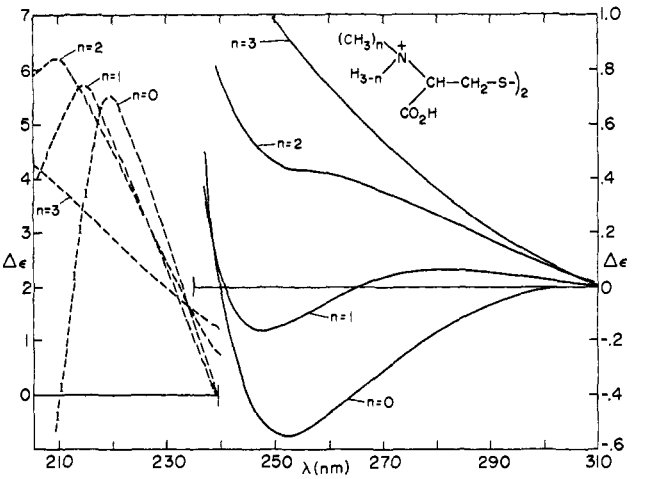

Figure 4. CD of N-methylated L-cystines in 1 *N* HCl.

Table IV. Circular Dichroism and Absorption Spectra of Alkyl Disulfides

Compd	Solvent	Δε	λ	λ_0[a]	ε	λ
17	*n*-C_7H_{16}	−4.8	290	262	290	290
		+3.7	243	228	110	234
		−30.6	203		4100	198
	CH_3CN	−5.0	292	261	310	289
		+2.7	243	231	130	242
		−11.0	213[b]		5040	213[b]
18	*n*-C_7H_{16}	+0.09	270	251	400	252
		−0.05	238	234		
	CH_3CN	+0.10	267	252	470	248
		−0.08	240	228		
	CH_3OH	+0.09	270	246	440	246
		−0.06	238	228		

[a] Crossover point where Δε = 0. [b] Shortest wavelength (nm) reliably measured.

Reflecting the opposite configuration at the asymmetric carbon, the carboxyl region CD of D-penicillamine disulfide (**25**) (Figure 5) is of opposite sign to **1**. In contrast, the disulfide region exhibits CD of like rather than opposite sign. The CD extremum at 262 in neutral solution is not accompanied by any maximum or shoulder in the absorption spectrum for **25**. Difference spectra of **25** *vs.* 2 equiv of L-valine or L-serine result in featureless absorption which increases in intensity from 310 to 240 nm (ϵ ~400) without exhibiting any maximum or shoulders, resembling the spectrum reported for another acyclic ditertiary disulfide, di-*tert*-butyl disulfide.[31]

Steric strain, evident in space-filling models of tertiary disulfides, accounts for the lack of a distinct long

(28) E. Abderhalden and E. Wybert, *Chem. Ber.*, 49, 2449 (1916).
(29) K. Bloch and H. T. Clarke, *J. Biol. Chem.*, 125, 275 (1938); G. Toennies, T. F. Lavine, and M. A. Bennet, *ibid.*, 112, 493 (1935).
(30) M. P. Schubert, *ibid.*, 111, 671 (1935).
(31) H. P. Koch, *J. Chem. Soc.*, 394 (1949).

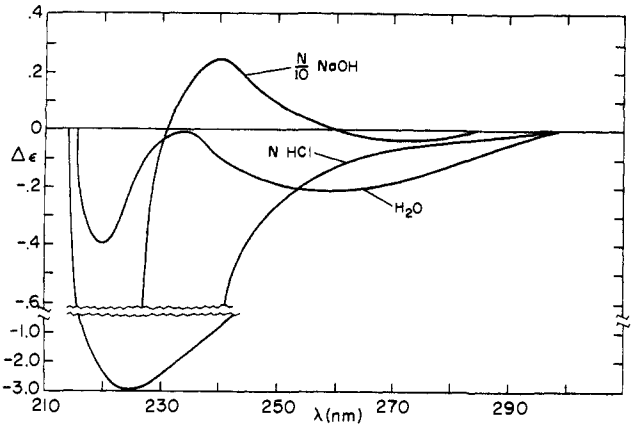

Figure 5. CD of D-penicillamine disulfide in aqueous solutions.

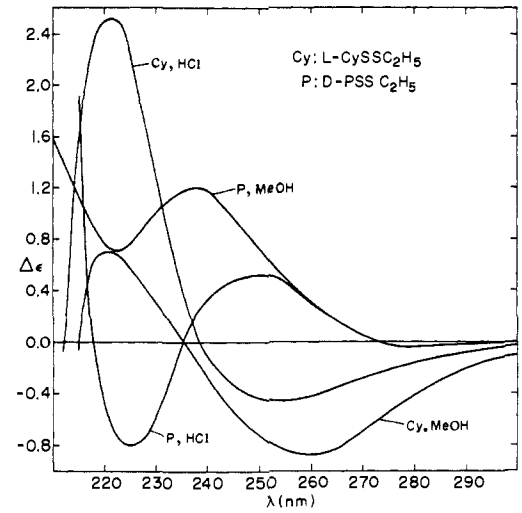

Figure 6. CD of mixed ethyl disulfides of L-cystine (**4**) and D-penicillamine (**26**) in 1 *N* HCl and methanol.

wavelength disulfide peak in their absorption spectra. Nonenantiomeric CD curves in this spectral region between **1** and **25** combined with nearly enantiomeric curves between the mixed disulfides **4** and **26** (Figure 6) suggest some interaction between the two halves of **25**, altering rotamer populations or even biasing disulfide screw sense.

The CD changes noted above for **1** in acid *vs.* neutral solution are mimicked in both L-cysteine mixed disulfides **4** and **5**. Upon an increase in the temperature from 25 to 49°, the magnitude of the CD extremum at 252 nm decreased an identical 17% for both **1** and **4**. These compounds exhibit uv spectral maxima nearly equal to **1**. **26** shows no clear maximum in acid, but in MeOH exhibits a distinct shoulder at *ca.* 245 nm, typical of a mixed tertiary–primary disulfide.[32]

The model alkyl disulfides **17** and **18** allow examination of the chiroptical properties of isolated disulfide chromophores: the former is a rigid molecule with a preferred (M) disulfide screw sense, and the latter is nonrigid with, presumably, little preferred screw sense. The uv and CD spectra of **17** reveal three transitions (Table IV). The 290-nm CD band arises from that transition which is red-shifted with decreasing disulfide dihedral angle. This red shift has been extensively characterized for model synthetic[32,33] and naturally occurring disulfides.[34] The second CD band at *ca.* 240 nm corresponds to the ultraviolet maximum at 243 nm, which is clearly defined in hydrocarbon solvents but appears only as a shoulder in more polar solvents. Such a second disulfide transition may be discerned in disulfide spectra reported elsewhere.[32,35,36] These long wavelength results for **17** in heptane are similar to those reported using isooctane as a solvent.[35] The third CD band extremum is quite distinct at 203 nm in heptane, and is associated with a disulfide transition which contributes to the uv maximum at 198 nm in that solvent. Short wavelength transitions have not been well characterized for other cyclic disulfides. A short wavelength dimethyl disulfide absorption maximum occurs at 196 nm in the vapor with a shoulder at 210 nm.[37] The dissymmetry ratio, $\Delta\epsilon/\epsilon$, is twice as great for the two bands at >230 nm (both magnetic dipole allowed) than for the shortest wavelength band near 200 nm.

Circular dichroism measurements of crystals of **1** and its hydrohalide salts, each prepared as specified for earlier X-ray diffraction analysis,[38,39] correlate CD sign and magnitude with known disulfide chirality. Single crystals of hexagonal **1** which has P disulfide chirality and *h* rotamer show positive CD in the region 310–280 nm. Transparent KBr pellets of the dihydrochloride (**27**) and dihydrobromide (**28**) of **1**, both with M disulfide chirality and solely *h* rotamer, yield $\Delta\epsilon = -1.6$ at 270 nm and $\Delta\epsilon = -1.7$ at 264 nm, respectively, with substantial positive CD near 230 nm. These CD results for **1**, **27**, and **28** qualitatively disagree in absorption and wavelength characteristics with one report,[40] but are in excellent quantitative agreement with others where $\Delta\epsilon = +2.0$ at 287 nm for **1** and $\Delta\epsilon = -1.9$ at 272 nm and +2.3 at 225 nm for **27**, all for crystals in KBr pellets.[41] These crystal spectra demonstrate that mutual cancellation of oppositely signed overlapping CD curves due to inherent dissymmetry and arising from two disulfide transitions is not complete for dihedral angles near 90°. Either the degeneracy is incomplete for these dihedral angles or the magnitudes of the oppositely signed CD contributions are unequal.

Discussion

Absence of Endocyclic Interactions. Due to apparent differences in the pmr spectra of L- and *meso*-cystine (ABX for the former, deceptively simple ABX for the

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(33) J. A. Barltrop, P. M. Hayes, and M. Calvin, *J. Amer. Chem. Soc.*, 76, 4348 (1954).
(34) R. Rahman, S. Safe, and A. Taylor, *Quart. Rev., Chem. Soc.*, 24, 208 (1970).
(35) M. Carmack and L. A. Neubert, *J. Amer. Chem. Soc.*, **89**, 7134 (1967).
(36) G. Claeson, *Acta Chem. Scand.*, **13**, 1709 (1959); L. Schotte, *Ark. Kemi*, **8**, 579 (1956).
(37) S. D. Thompson, D. G. Carroll, F. Watson, M. O'Donnell, and S. P. McGlynn, *J. Chem. Phys.*, **45**, 1367 (1966).
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(39) (a) L. K. Steinrauf, J. Peterson, and L. H. Jensen, *J. Amer. Chem. Soc.*, **80**, 3835 (1958); (b) J. Peterson, L. K. Steinrauf, and L. H. Jensen, *Acta Crystallogr.*, **13**, 104 (1960).
(40) P. C. Kahn and S. Beychok, *J. Amer. Chem. Soc.*, **90**, 4168 (1968).
(41) A. Imawishi and T. Isemura, *J. Biochem. (Tokyo)*, **65**, 309 (1969); N. Ito and T. Takagi, *Biochim. Biophys. Acta*, **221**, 430 (1970).

latter), it has been suggested[42] that in acid solution the former does possess a conformation stabilized by intramolecular forces[3] and that analysis of rotamer populations from vicinal coupling constants is not applicable to cystines. These suggestions[3,42] are contradicted by the similarity of the vicinal coupling constants of **1** and those of **3** and **4**, where intramolecular interactions of the type proposed cannot exist. An alternative explanation of the observed differences, one predicated on differential shielding effects, in no way precludes estimates of rotamer populations in those cases where ABX spectra are apparent.[43]

An additional argument against the existence of endocyclic interactions arises from consideration of the circular dichroism spectra of **1**, **4**, and **5**. The $\Delta\epsilon$ values for **4** and **5** are approximately one-half of those of **1** in acid and in neutral solution. Were there strong endocyclic interactions of **1**, the CD spectrum should so indicate by being more intense than twice that of **4** or **5**, due to restricted molecular mobility.[4]

A third insight arises from the acidity constants from ammonium deprotonations in **1**. The pK_a difference of 1.0 log unit (see Experimental Section) is within the range expected for two charged centers separated by seven single bonds with free rotation; a similar difference of 1.1 log units is observed for 1,6-diaminohexane.[44] Titration measurements are sensitive to intramolecular interactions, and normal pK_a values for **1** indicate that no significant fraction of the ammonium groups of **1** or **25** is involved in intramolecular interactions.

The absence of endocyclic interactions weakens the argument for any measurable contribution to optical activity from inherent dissymmetry induced by significant biasing of one disulfide screw sense over another in **1** and its derivatives. Exhaustive examination of molecular models also fails to reveal cause for biasing of one screw sense. The discussion that follows is consistent with and supports negligible contributions to optical activity from inherent dissymmetry in simple derivatives of L-cystine.

Correlation of Rotamer Populations with Optical Activity. There is a striking parallelism between the pmr vicinal coupling constant difference and optical activity in the long wavelength disulfide absorption bands. Comparison of the coupling constant difference $J_{AC} - J_{BC}$ in Table I with the sign and magnitude of $\Delta\epsilon$ at >240 nm in Table III and Figure 4 for the successively N-methylated cystines indicates a proportional relation between them. The most negative coupling constant difference is associated with the most negative CD (**1**), nearly equal vicinal coupling constants attend a weak CD curve with both negative and positive regions (**12**), and the most positive coupling constant difference is associated with the most positive CD (**14**).

Rotamer populations may be estimated if assigned values of J_T and J_G remain constant for **1** and **12–14**. Values of J_T = 12.8 and J_G = 2.5 Hz are consistent with several studies on amino acids and derivatives.[14] For each compound, the mole fraction difference $t - g$ may be found from eq 1 and the population of rotamer h calculated from eq 2. The sum of the three mole fractions $t + g + h = 1$ so that each may be evaluated. The results of such calculations from the vicinal coupling constants listed in Table I for N-methylated cystines are presented in Table V.

Table V. Rotamer Populations and CD of Acid Solutions of N-Methylated L-Cystines, $(CH_3)_nNH_{3-n}CH(COOH)CH_2S-_2$

		1	**12**	**13**	**14**
			n =		
		0	1	2	3
		Rotamer population			
Rotamer mole fraction	t	0.50	0.26	0.08	0.09
	g	0.18	0.27	0.51	0.91
	h	0.32	0.47	0.41	0.00
		Circular dichroism			
255 nm	$\Delta\epsilon_{obsd}$	−0.53	−0.10	+0.42	+0.88
	Calcd[a]	−0.53	−0.11	+0.41	+0.87
285 nm	$\Delta\epsilon_{obsd}$	−0.11	+0.05	+0.21	+0.28
	Calcd[b]	−0.11	+0.05	+0.21	+0.28

[a] Calculated at 255 nm with $\Delta\epsilon_t = -1.4$, $\Delta\epsilon_g = +1.1$, and $\Delta\epsilon_h = -0.1$. [b] Calculated at 285 nm with $\Delta\epsilon_t = -0.45$, $\Delta\epsilon_g = +0.35$, and $\Delta\epsilon_h = +0.15$. Results refer to room temperature, about 25°.

Successive N-methylation of **1** increases the size of the ammonium group and the population of rotamer g as shown in Table V. For **14** where $n = 3$, rotamer g is the most favored. For $n \leq 2$, rotamer h is well populated. This conclusion is consistent with the lack of temperature dependence of the vicinal coupling constant sum and the exclusive occurrence of rotamer h in crystals of L-cystine,[38] L-cystine·2HCl,[39a] L-cystine·2HBr,[39b] *N,N'*-diglycyl-L-cystine,[45] cysteine·HCl,[46] the urea adduct of cysteine ethyl ester·HCl,[47] and glutathione.[48]

Since the contribution from inherent disulfide disymmetry cancels for equally populated screw senses, for a given wavelength the magnitude of the observed CD may be expressed as the weighted sum of contributions from each rotamer

$$\Delta\epsilon = t\Delta\epsilon_t + g\Delta\epsilon_g + h\Delta\epsilon_h \qquad (3)$$

where $\Delta\epsilon_t$ is differential molar absorptivity of rotamer t, and similarly for $\Delta\epsilon_g$ and $\Delta\epsilon_h$. If we assume that these last three quantities are unaltered by successive N-methylation of cystine, their values may be calculated by solving three simultaneous equations (3) for three of the four compounds of Table V, with the fourth compound providing a check on the validity of the analysis. At 255 nm, the constants obtained are as follows: $\Delta\epsilon_t = -1.4$; $\Delta\epsilon_g = +1.1$; and $\Delta\epsilon_h = -0.1$. Utilization of these constants permits cal-

(42) J. A. Glasel, *J. Amer. Chem. Soc.*, **87**, 5472 (1965).

(43) The deceptively simple ABX pmr spectrum of *meso*-cystine may be rationalized in terms of a spectrum wherein $\Delta\nu_{A-B} < J_{AB}$.[42] $\Delta\nu_{A-B}$ for **3** or **4** is approximately 3–4 Hz less than $\Delta\nu_{A-B}$ for **1** (Table I). Assuming a major portion of this difference is due to an increase in intrinsic diastereotopism induced by the addition of a second chiral center in **1** relative to **3** or **4**, and reversing the sign of this contribution from the second chiral center, *i.e.*, generating *meso*- rather than L-cystine, we conclude $\Delta\nu_{A-B}$ for *meso*-cystine would be in the range 4–6 Hz at 100 MHz, small enough to satisfy the conditions required for a deceptively simple spectrum.[19]

(44) D. H. Everett and B. R. W. Pinsent, *Proc. Roy. Soc., Ser. A*, **215**, 416 (1952).

(45) H. L. Yakel, Jr., and E. W. Hughes, *Acta Crystallogr.*, 7, 291 (1954).

(46) R. R. Ayyar, *Z. Kristallogr.*, **126**, 227 (1968). Exclusivity of rotamer h does not apply to L-cysteine as rotamers h and g have been found for the two different conformations in one asymmetric unit: M. M. Harding and H. A. Long, *Acta Crystallogr., Sect. B*, 24, 1096 (1968).

(47) D. J. Haas, *Acta Crystallogr.*, **19**, 860 (1965).

(48) W. B. Wright, *ibid.*, **11**, 632 (1958).

culation of $\Delta\epsilon$ values by eq 3 which are compared with observed values for the four compounds of Table V. The agreement between calculated and observed values is excellent for all four compounds. Since the $\Delta\epsilon$ values at 255 nm for the four cystines of Table V are almost identical with the net $\Delta\epsilon$ values observed at >240 nm, similar numerical constants are obtained for the net CD of each rotamer.

Since at least two disulfide transitions occur at >240 nm, an analysis similar to the above was also performed at 285 nm. At 285 nm, the constants evaluated are: $\Delta\epsilon_t = -0.45$; $\Delta\epsilon_g = +0.35$; and $\Delta\epsilon_h = +0.15$. Agreement between four calculated and observed values at 285 nm in Table V is perfect. As at 255 nm, the sum of the three constants is nearly zero, indicating that in **1** and derivatives equal populations over all three rotamers would yield nearly no disulfide optical activity at >240 nm. Comparison of the constants obtained at the two wavelengths suggests that $\Delta\epsilon_t$ and $\Delta\epsilon_g$ are negative and positive, respectively, throughout the long wavelength disulfide region, while $\Delta\epsilon_h$ is positive for the longer wavelength and negative for the shorter wavelength transitions at >240 nm. Thus rotamer *h* with substituents in both positions gauche to sulfur yields the lowest CD, due at least in part to cancellation of postive and negative CD components of nearly degenerate transitions. If some molecule contained a disulfide bond with no bias of screw sense and predominantly rotamer *h*, its optical activity could increase with an increase in temperature as transformation to the more active rotamers *t* or *g* occurs. On the basis of first-order symmetry arguments, the sector rule for an asymmetrically perturbed disulfide chromophore is an octant rule.

Comparison of the composite long wavelength disulfide CD sign and magnitude changes of Figure 3 with Figure 4 and Table III suggests that bulkier side chains of alcoholic solvents cause alkyl ester dihydrochlorides of **1** to undergo a transformation similar to that of **1** upon successive N-methylations. Solvation of ammonium groups is an essential feature for interpretation of their properties.[49] Increasing size and branching of the alcoholic alkyl moiety increases the effective size of the solvated ammonium group, thereby leading to an increasing preference for rotamer *g* and the resultant more positive disulfide CD.

Correlation of Screw Sense with Optical Activity. Assignment of the CD sign to disulfide screw sense in **1** and derivatives requires consideration of the crystal results, since both screw senses are present in nearly equal amounts in solution. It is also desirable to subtract the contribution to optical activity due to asymmetric perturbation of the disulfide chromophore by the specific rotamer present. All crystals so far examined contain only rotamer *h*. Addition of the quantitative crystal CD results on left-handed L-cystine·2HCl and right-handed L-cystine[41] cancels the opposed screw sense contributions and yields the CD sum for two *h* rotamers. In striking agreement with the conclusions made from solutions, the crystal results indicate that the CD for rotamer *h* is a small positive value near 290 nm and negative at <280 nm.[50] Subtraction of the CD for a disulfide chromophore asymmetrically perturbed by rotamer *h* from the crystal results yields the conclusion that a disymmetric disulfide bond in L-cystine gives a net or long wavelength positive CD at >250 nm for P disulfide chirality and a negative CD for M disulfide chirality. At the dihedral angles occurring in the crystals, the dissymmetric disulfide bond gives a CD extremum near 280 nm with $\Delta\epsilon = \pm 2.0$. Since this value is only 50% greater than possible rotameric contributions to optical activity, assignments of an exclusive screw sense to naturally occurring cystine derivatives that exhibit $\Delta\epsilon < 1$ do not seem to be warranted without further analysis.

Consistent conclusions have been obtained to support the association of a specific CD sign with chirality of dissymmetric disulfide bonds in a number of compounds not directly related to **1**. Circular dichroism spectra associated with the two longer wavelength transitions are opposite in sign for **17** and for other 1,2-dithianes with two β,[35] one β,[51] two α,[52] and one α[51] chiral center(s). For each of these 1,2-dithianes M chirality is associated with a negative CD at *ca.* 280–290 nm and a positive CD at *ca.* 240 nm. The correlation of M chirality with a negative long wavelength CD also appears general for disulfide dihedral angles less than 55–60°.[52–55] Where identified[55] the second CD band is opposite in sign to the first and, as the disulfide dihedral angle diminishes, the rotational strengths of both transitions decrease.

Malformin. The tricyclic pentapeptide malformin A (**27**) (cyclo-L-isoleucyl-D-cysteinyl-L-valyl-D-cysteinyl-D-leucyl), an acylated and amidated derivative of D-cystine, undergoes solvent-induced conformational changes associated with alterations in biological activity.[56] This section demonstrates the utility of disulfide CD as a sensitive indicator of structure and monitor of transformations. That the disulfide bond is strained as determined by polarography[56c] is difficult to confirm by ultraviolet spectra, as no maximum is evident in Figure 7. However, the first CD band at 280 nm suggests that the dihedral angle is less than 90° but greater than the 60° found in 1,2-dithianes. The sign pattern and magnitudes for the 280-nm negative and 235-nm positive disulfide CD bands suggest a predominantly M screw sense. The shortest wavelength CD band at 215 nm in Figure 7 is assigned to carboxamide transitions. The magnitudes for the two longer wavelength CD bands of Figure 7 are the largest, certainly identifiable as due to disulfide in a polypeptide.

When treated with dilute Na_2CO_3, the prevailing conformer of **27**, MaI, undergoes transformation to an

(49) T. P. Pitner and R. B. Martin, *J. Amer. Chem. Soc.*, **93**, 4400 (1971), and references cited therein.

(50) Ultraviolet absorption spectra of crystals of **1** and **27** strongly resemble their solution spectra, indicating an absence of major wavelength shifts which might weaken comparison of solution and crystal CD spectra. The following conclusions associating a positive CD with P disulfide chirality and our CD results on crystals were presented orally at the Seventh International Congress of Biochemistry in Tokyo in August, 1967.

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(53) A. F. Beecham and A. M. Mathieson, *Tetrahedron Lett.*, 3131 (1966).

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(55) R. Nagarajan, N. Neuss, and M. M. Marsh, *ibid.*, **90**, 6518 (1968).

(56) (a) N. Takahashi and R. W. Curtis, *Plant Physiol.*, **36**, 30 (1961); (b) S. Marumo and R. W. Curtis, *Phytochemistry*, 1, 245 (1961); (c) K. Isono and R. W. Curtis, *ibid.*, **3**, 277 (1964); (d) K. Anzai and R. W. Curtis, *ibid.*, 4, 263 (1965); (e) K. Anzai and R. W. Curtis, *ibid.*, 4, 713 (1965).

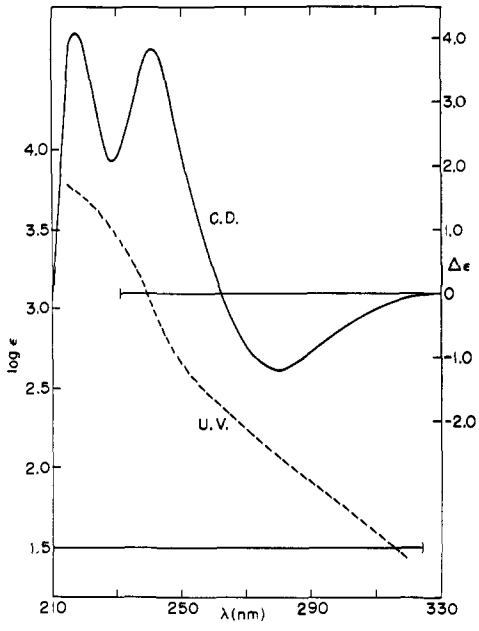

Figure 7. CD and absorption spectra of malformin A, conformation I, in trifluoroethanol.

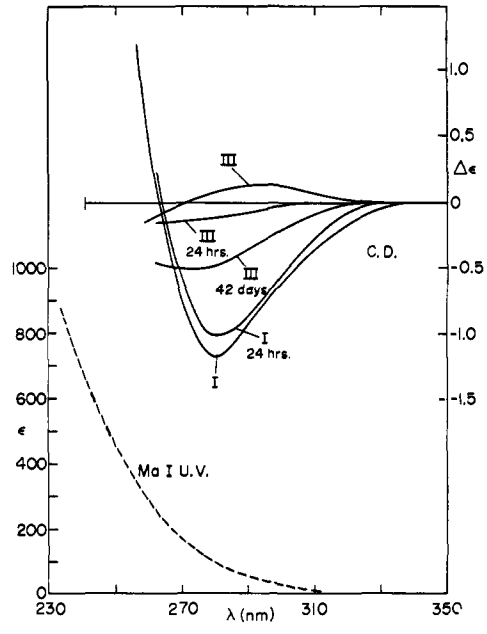

Figure 8. CD of malformin A, conformations I and III, in trifluoroacetic acid and the changes wrought by prolonged standing.

even less soluble conformer, MaIII.[56e] The corresponding change from negative to positive long wavelength disulfide CD is shown in Figure 8. Upon prolonged standing in trifluoroacetic acid, both conformers MaI and MaIII adopt yet a third conformation with an intermediate negative CD. Thus the M chirality forced upon the disulfide bond by an ordered secondary structure is lost in competitive hydrogen bonding solvents.

Experimental Section

Methods. Microanalyses were performed by Galbraith Laboratories, Knoxville, Tenn. Melting points (heated oil bath) and boiling points are uncorrected. D-line rotations were obtained employing a Rudolph polarimeter, and D-line refractive indices were recorded with an Abbé refractometer. Infrared spectra were determined on a Perkin-Elmer Model 337 using KBr matrices for solids and NaCl disk smears for liquids. Glc columns were run on a Varian Aerograph Model 90-P equipped with an E. H. Sargeant Model S.R. recorder. Ultraviolet spectra were recorded on a Cary 14 spectrophotometer, and CD spectra were recorded on a Durrum–Jasco Model ORD/UV-5 automatic recording spectropolarimeter fitted with a circular dichroism attachment. Spectroquality solvents were used when commercially available. Other solvents were purified by known techniques.[57] Optical densities of solutions for CD analysis were routinely kept below 1.5 at shorter wavelengths. All spectra were multiply checked for precision using various pathlength cells. No significant deviations from Biots law were found in any instance. Reproducible CD magnitudes of L-cystine salts were calculated from the weighed amounts present in KBr pellets of measured volume and thickness.

Routine pmr spectra were recorded on a Varian A-60 instrument. Coupling constants and chemical shifts tabulated in the Results were obtained employing a Varian HA-100 spectrometer. Peaks were individually assigned (±0.05 Hz) with the V 4315 frequency counter when it was found that standard HA mode calibration was faulty due to nonlinear sweep over a 100-Hz range. AB and ABX calculations were done routinely.[23] An ALGOL translation of LAOCOON II run on the University of Virginia Burroughs V 5500 computer calculated (rms < 0.09) ABC spectra. Temperature-dependent 100-MHz spectra were calibrated for temperature (±1.5°) by routine methanol or ethylene glycol procedures.

Substituted cysteine pmr solutions were prepared as 1.0 mequiv of cysteine/1.0 ml of solvent, that is, for *S*-methylcysteine, 0.4 mmol/0.4 ml, and for L-cystine, 0.2 mmol/0.4 ml. Stock solutions of either DCl or NaOD were mixed with D_2O to give solvent, assuring the desired solute ion form (20% excess DCl was used for cations). By convention, internal 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt (TSS) is used as a D_2O reference. The concentration of TSS needed to assure a stable 100-MHz mode lock (5–15%) interferes with isoelectric point solubility in many instances, however, and may alter modes of solute hydrogen bonding. For this reason, no internal standard was used. The HDO peak resulting from proton exchange and trace amounts of protons in the stock solutions was used as internal lock quite successfully.

Acidity constants for ammonium deprotonations of **1**, **4**, **5**, and **25** were determined at 5 m*M* concentration and 25° on a Radiometer Titration–Titrigraph combination. Overlapping acidity constants for **1** and **25** were resolved by a previously described method.[58] At 0.10 *M*, ionic strength values for **1** are $pK_3 = 7.95$ and $pK_4 = 8.95$. The average of this pair of values nearly equals the identical experimental values of $pK_2 = 8.40$ obtained for both **4** and **5**. For **25**, at 0.15 ionic strength, $pK_3 = 7.60$ and $pK_4 = 8.70$.

Materials. General. L-Cysteine (**2**), L-cysteine hydrochloride monohydrate, *S*-methyl-L-cysteine (**3**), L-cystine (**1**), *meso*-cystine, glutathione (reduced) (**6**), *S*-methylglutathione (**7**), glutathione (oxidized) (9), D-penicillamine (reduced), L-cystinylbisglycine (**11**), L-cystinylbis-L-alanine monohydrate (**20**), L-cystinylbis-L-leucine (**21**), and L-cystinyl di-*tert*-butyl ester dihydrochloride (**24**) were purchased from commercial sources, highly purified. Commercial *N*,*N*′-diglycyl-L-cystine dihydrate (**10**) was obtained chromatographically inhomogeneous and was recrystallized from water–propanol to yield homogeneous crystalline peptide. L-Lithium cystinate[59] was obtained by the addition of 180 ml of acetonitrile to a filtered solution of 3.6 g of **1** in 60 ml of 0.40 *M* LiOH in absolute ethanol. After drying, the crystalline precipitate had mp >280° and a sharp NH_2 doublet in the ir at 3500 and 3350 cm^{-1}. L-Cystine dihydrochloride (**27**), mp 199–200° dec, crystallized as needles from a solution of **1** in warm 6 *N* HCl. L-Cystine dihydrobromide (**28**), mp 202–203° dec, was deposited as long needles from a solution of **1** in 48% HBr.

L-Cystine dimethyl ester dihydrochloride (**23**) (mp 169–172° dec, $[\alpha]^{25}D$ +53.8° (*c* 0.546, EtOH), −45.7° (*c* 0.464, MeOH); lit.[28] mp 166° dec, $[\alpha]^{20}D$ +49.89° (EtOH), −38.0° (MeOH)) and L-cystine diethyl ester dihydrochloride (**22**) (mp 186–187.5° dec, $[\alpha]^{25}D$ −62.4° (*c* 0.517, MeOH), −42.1° (*c* 0.505, H_2O); lit.[28] mp 188° dec, $[\alpha]^{20}D$ +40.08° (EtOH), −54.06° (MeOH), $[\alpha]D$ 48.10° (H_2O)) were pre-

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pared by the Fischer technique. Ir, 60-MHz pmr, and microanalyses were consistent with the assigned structures. *N,N'*-Diformyl-L-cystine was prepared following literature procedures[60] exactly: after two recrystallizations from H_2O, mp 186–188°, $[\alpha]^{27}D$ −147.7° (*c* 0.49, 1 *N* NaOH); lit. mp 187–188°, $[\alpha]^{25}_{546}$ −162.1° (*c* 1.0, 1 *N* NaOH). D-Penicillamine disulfide (**25**) was prepared by the Fe^{3+}-catalyzed air oxidation[61] of commercially available D-penicillamine $[\alpha]^{23}D$ −60° (*c* 1.00, 1 *N* NaOH). Following two precipitations from H_2O by addition of EtOH, analytic material was obtained: mp 206–208°, $[\alpha]^{26}D$ +104.6° (*c* 1.02, H_2O), lit.[61] mp 204–205°, $[\alpha]^{23}D$ +118° (*c* 0.8, H_2O).

Methylated Cystines. *N,N'*-Dimethyl-L-cystine (**12**) was prepared by the Fe^{3+}-catalyzed air oxidation of *N*-methyl-L-cysteine[62] in aqueous solution. *N*-Methyl-L-cysteine was obtained by Na–NH_3 reduction of the condensation product of L-cysteine and formaldehyde, 4-carboxythiazolidine, mp 197–198.5°, lit.[62] mp 196–197°. The crude disulfide **12** was precipitated from a neutral H_2O solution by addition of EtOH. The flocculent precipitate was centrifuged and dried, mp 206–210°. Crude disulfide (540 mg) was suspended in 4 ml of H_2O and dissolved by addition of a minimal amount of 1 *N* NaOH. The solution was filtered and chilled overnight, following addition of 1 *N* HCl to pH 6–8. Crystalline material (415 mg) was recovered, mp 213–216° dec, after drying *in vacuo*. Repeated purification by this procedure yielded identically melting material. An analytic sample was homogeneous in DCl by 100-MHz pmr. Microanalysis was most consistent with a monohydrate structure.

Anal. Calcd for $C_8H_{16}N_2O_4S_2 \cdot H_2O$: C, 33.55; H, 6.33; N, 9.78; S, 22.39. Found: C, 34.08; H, 6.09; N, 9.79; S, 22.47.

N,N,N',N'-Tetramethyl-L-cystine (**13**) was prepared by a route analogous to that used for preparation of **12**. Material previously cited as *N,N,N',N'*-tetramethyl-L-cystine obtained by reductive formylation of L-cystine[20] has been shown to be 3-methyl-4-carboxythiazolidine.

A. 3-Methyl-4-carboxythiazolidine hydrochloride (**16**) was prepared by the condensation of *N*-methyl-L-cysteine hydrochloride with formaldehyde (1:1 ratio).[29] The crude product in EtOH was decolorized with charcoal and precipitated from EtOH upon addition of Et_2O. Repeated purification by EtOH–Et_2O recrystallization yielded material with mp 180–182° dec, lit.[29] mp 180–181°.

B. 3-Methyl-4-carboxythiazolidine was prepared by following the Bowman and Stroud[20] procedure exactly. **1** (3.60 g, 0.015 *M*) and 10% Pd/C (3.60 g) were suspended in 150 ml of H_2O to which 10.3 ml of 37% formaldehyde solution (0.120 *M*) had been added. The mixture was hydrogenated on a Parr apparatus at 22 psi. After 5 hr the pressure drop was 4.0 psi and after 29 hr the pressure drop stabilized at only 4.2 psi. The solution was removed from the Parr apparatus and heated to boiling, and the Pd/C filtered. The filtrate was concentrated and the remaining yellow gum taken up in hot H_2O. The solution was decolorized with charcoal and, following filtration, the filtrate was again concentrated at reduced pressure. The resulting gum was allowed to partially crystallize slowly (88 days). Only in this manner was the literature product (lit.[20] specifies "oxidative exposure to air") obtained. The crystals were extracted with boiling CP acetone and recrystallized from acetone (or more easily from acetone–*n*-hexane) yielding analytic material (490 mg): mp 102–104.5° after drying, $[\alpha]^{27.4}D$ −152° (*c* 0.566, H_2O), lit.[20] mp 105–107° (soften 90°), $[\alpha]^{20}D$ −146° (H_2O), with no yield stated. The 100-MHz spectrum of this material in DCl solution was superimposable with that of 3-methyl-4-carboxythiazolidine hydrochloride in DCl solution. The ultraviolet spectrum in H_2O showed only a weak shoulder at 235 nm (ϵ 70 calculated on the basis of thiazolidine, mol wt = 147.2). Upon NaOH addition, the extinction coefficient at 240 nm increased to >1000, indicative of thiazolidine hydrolysis and thiolate anion absorption. In 1 *N* HCl the CD spectra of the products from A and B were also completely superimposable.

Anal. Calcd for $C_5H_9NO_2S$: C, 40.80; H, 6.16; N, 9.52; S, 21.78. Found: C, 40.79; H, 6.64; N, 9.44; S, 21.69. Calcd for $C_{10}H_{20}N_2O_4S_2$ (*N,N,N',N'*-tetramethyl-L-cystine): C, 40.52; H, 6.80; N, 9.45. Found:[20] C, 40.7; H, 6.7; N, 9.6.

From the identity of the pmr, uv, and CD spectra in acid, the Bowman–Stroud[20] material $[\alpha]^{27.4}D$ −152°, *i.e.*, $[M]^{27.4}D$ −224° (H_2O) must be reassigned as the free base of 3-carboxyl-4-L-thiazolidine hydrochloride, $[\alpha]^{24}D$ −119.2°,[29] $[\alpha]^{27}D$ −123.2°,[61] *i.e.*, $[M]^{24}D$ −218.9°, $[M]^{27}D$ −226.3°.

C. 3-Methyl-4-carboxythiazolidine hydrochloride (mp 180–181° dec, 9.18 g, 0.05 *M*) was slowly dissolved in liquid ammonia (250 ml) containing a trace of H_2O (2.4 ml). Sodium was added until the transient blue color persisted for >30 min. After portionwise addition of NH_4Cl (11 g), the solvent was allowed to evaporate. Final traces of NH_3 were removed *in vacuo* and the resultant solid was taken up in H_2O (250 ml). This solution was made strongly acidic with concentrated HCl, then concentrated to a thick oil *in vacuo*. The oil was extracted with absolute EtOH (400 ml) and the resulting solution was chilled. Precipitated NH_4Cl was removed by filtration. The EtOH was concentrated to 150 ml and the removal of NH_4Cl was repeated. The filtrate was decolorized with charcoal and concentrated to a residual oil. This oil, crude *N,N'*-dimethyl-L-cysteine, was dissolved in H_2O (100 ml) which was brought to pH 8–9 with concentrated NH_4OH. Trace amounts of $FeCl_3$ were added and the solution was aerated for 18 hr. Following oxidation, the solution was brought to pH 5–6 with HOAc and again decolorized. Addition of an equal volume of absolute EtOH to a water solution of the disulfide followed by addition of 10 vol of CP acetone proved a costly but sufficient method of purification by crystallization. Following three such recrystallizations, analytically pure *N,N,N',N'*-tetramethyl-L-cystine (**13**) (225 mg) was isolated, mp 192.5–193° dec.

Anal. Calcd for $C_{10}H_{20}N_2O_4S_2$: C, 40.52; H, 6.80; N, 9.45; S, 21.64. Found: C, 40.34; H, 6.87; N, 9.24; S, 21.84.

N,N,N,N',N',N'-Hexamethyl-L-cystine (L-cystine betaine (**14**)) was prepared following an improved method of exhaustive methylation of L-cystine using dimethyl sulfate.[20] After decolorization of the water solution, acetone was used to precipitate the betaine. Water–acetone recrystallization yielded analytic material, mp 170–171° dec, $[\alpha]^{25.2}D$ +338° (*c* 0.553, H_2O), and $[\alpha]^{25.4}D$ +261° (*c* 0.491, 1 *N* HCl).

Anal. Calcd for $C_{12}H_{24}N_2O_4S_2$: C, 44.42; H, 7.45; N, 8.63. Found: C, 44.11; H, 7.52; N, 8.47.

Mixed Disulfides. L-Cysteine hydrochloride monohydrate (1.75 g, 0.01 *M*) and β-mercaptoethanol (7.81 g, 0.10 *M*) (Eastman) in H_2O (20 ml) were cooxidized at pH 8–9 (1.0 ml of concentrated NH_4OH added) by aeration with trace $FeCl_3$ catalysis. After 20 hr the solution was filtered free of contaminant **1** and the unsymmetrical water-soluble disulfide **5** was precipitated by addition of 4 vol of CP acetone, 880 mg, mp 169–170.5° dec. The solid was dissolved in H_2O (40 ml), EtOH (40 ml) was added, and the turbid solution was filtered. Et_2O (100 ml) was added to the filtrate yielding, after drying *in vacuo*, 2-hydroxyethylthio-L-cysteine (**5**), 315 mg, mp 169–171° dec, lit.[63] mp 161–162° dec. Ascending paper chromatography (6:1.5:2.5 *n*-BuOH–HOAc–H_2O) indicated homogeneity, R_f 0.37.

Anal. Calcd for $C_5H_{11}NO_3S$: C, 30.44; H, 5.62; N, 7.10; S, 32.51. Found: C, 30.70; H, 5.79; N, 6.89; S, 32.31.

S-Ethylthio mixed disulfides were prepared by the addition of freshly distilled *S*-ethyl thioethyl sulfinate (EtSOSEt), bp 39–41° (0.2–0.35 mm), $n^{26.5}D$ 1.5241, lit.[64] bp 67° (0.5 mm), $n^{25}D$ 1.5244, to the appropriate mercaptan at neutral pH.

S-Ethylthio-L-cysteine (**4**) formed immediately as platelets upon slow addition of EtSOSEt (1.51 g, 120% excess) to a solution of L-cysteine hydrochloride monohydrate (1.75 g, 0.01 *M*) in H_2O (15 ml) brought to pH 6.35 by addition of 6% $NaHCO_3$. The platelets were filtered, washed, and dried *in vacuo*, 1.10 g (61%), mp 204–206° dec.

Anal. Calcd for $C_5H_{11}NO_2S_2$: C, 33.13; H, 6.17; N, 7.73; S, 35.38. Found: C, 33.23; H, 6.26; N, 7.59; S, 35.53.

EtSOSEt (455 mg, 120% excess) was added to a stirred solution of 447 mg of D-penicillamine in H_2O (10 ml). The solution was stirred 2 hr and the solvent then removed *in vacuo*. The resultant solid was dissolved in absolute EtOH (20 ml), then precipitated by slow addition of Et_2O. The gelatinous precipitate was washed well with Et_2O and dried *in vacuo* to give *S*-ethylthio-D-penicillamine, (**26**), 541 mg (86%), mp 170–172° dec.

Anal. Calcd for $C_7H_{15}NO_2S_2$: C, 40.16; H, 7.22; N, 6.69; S, 30.64. Found: C, 39.95; H, 7.43; N, 6.45; S, 30.46.

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Aliphatic Disulfides. (+)-(*S,S*)-Bis(2-methylbutyl) disulfide was synthesized from commercial "active amyl" alcohol, *S*, in three steps.

A. (*S*)-2-Methylbutyl *p*-Toluenesulfonate. "Active amyl" alcohol (10.0 g, 0.113 mol), $[\alpha]^{24}D$ −4.68° (0.5 dm tube, neat), lit.[65] $[\alpha]^{28}D$ −4.93° (1 dm tube, neat), was tosylated in 75 ml of pyridine (distilled from NaOH) with 33.2 g (0.175 mol) of toluenesulfonyl chloride (recrystallized from Et_2O). After standard work-up the tosylate was distilled, 24.55 g (81%), bp 142–147° (0.6 mm).

B. (+)-(*S*)-2-Methylbutyl Thiocyanate. The above tosylate in 40 ml of freshly distilled DMSO was added dropwise with stirring to a solution of 19.5 g (100% excess) of KSCN in 75 ml of DMSO held at 95°.[66] The mixture was stirred at 90° for 3.5 hr, allowed to cool to room temperature, and then poured into 400 g of ice water. After stirring for 30 min the solution was filtered and the filtrate was extracted with three 50-ml portions of Et_2O. The Et_2O was dried and stripped, and the residue distilled, bp 80–84° (14 mm), 8.15 g (62.5%). The ir was free of –OH stretch and a –SCN band at ~2150 cm^{-1} was present, $[\alpha]^{24.8}D$ +33.0° (*c* 1.92, EtOH).

C. (+)-(*S,S*)-Bis(2-methylbutyl) disulfide was prepared from the thiocyanate by oxidative hydrolysis.[67,68] The above thiocyanate (8.15 g) was refluxed in 45 ml of 2 *N* NaOH for 12 hr. The solution was cooled, acidified by pouring over 10.0 ml of concentrated HCl in 100 g of ice and water, and extracted with three 100-ml portions of Et_2O. The Et_2O was dried and stripped and the residue distilled (20 cm Vigreaux), 3.8 g (58%), bp 128° (13 mm). The disulfide was homogeneous by glc on 5-ft Porapak, SE -30, and 15% Carbowax 20M on firebrick columns, $[\alpha]^{24.8}D$ +97.8° (0.5 dm tube, neat). The lit.[69] value for active amyl disulfide of bp 120–122° (10 mm) ($[\alpha]^{20}D$ +72.48° (neat)) was determined on disulfide originating without racemization from active amyl alcohol of $[\alpha]^{18}D$ −3.35°. Assuming Whitmore's standard,[65] the lowest optical purity of the disulfide obtained here is (−3.35°/−4.93°) × (+97.8°/+72.48°)100 ≧ 91%. No correction for optical purity is made in the recorded CD spectra.

Anal. Calcd for $C_{10}H_{22}S_2$: C, 58.19; H, 10.74; S, 31.07. Found: C, 58.22; H, 10.90; S, 31.30.

(−)-(9*S*,10*S*)-*trans*-Hexahydro-2,3-benzodithiin was synthesized from (+)-(*S,S*)-*trans*-1,2-cyclohexanedicarboxylic acid in six steps in an overall yield of 14%. The starting diacid was obtained by ethanolysis–esterification of *cis*-hexahydrophthalic anhydride (Eastman) followed by base-catalyzed cis to trans epimerization and saponification, then acidification, mp 226–228.5°, lit.[70] 227–229°. The diacid was resolved following Applequist and Werner[71] exactly to yield diacid with mp 182–185°, $[\alpha]^{24}D$ +20.84° (*c* 5.0, acetone), lit. mp 179–183°, $[\alpha]^{20}D$ +18.2° (acetone),[72] mp 183–185°, $[\alpha]^{30}D$ +22.3° (*c* 5.3, acetone),[71] and $[\alpha]^{25}D$ +20.9° (*c* 3.5, acetone).[73]

A. (+)-(*S,S*)-*trans*-1,2-Bis(hydroxymethyl)cyclohexane was obtained in 94% crude yield by $LiAlH_4$ reduction of the diacid, mp 59–62°, lit.[71] mp 63–64°.

B. (+)-(*S,S*)-*trans*-1,2-Bis(hydroxymethyl)cyclohexane Di-*p*-toluenesulfonate. Diol (7.20 g, 0.05 mol) in 40 ml of ice-cold pyridine (distilled from NaOH) was added over 25 min to 22.9 g of recrystallized *p*-toluenesulfonyl chloride (20% excess) at −5° with rapid stirring. The reaction mixture was refrigerated for 2 hr, then poured into 250 g of ice and water. The precipitate was filtered, washed with cold water, and dried *in vacuo*, 19.3 g (80%). One recrystallization from MeOH gave a lead crop of 10.2 g, mp 110–111.5°, after drying *in vacuo*, $[\alpha]^{25}D$ +26.3° (*c* 2.51, C_6H_6), lit.[73] mp 109–109.7°, $[\alpha]^{28}D$ +25.0° (*c* 5.0, C_6H_6).

C. (+)-(*S,S*)-*trans*-1,2-Bis(bromoethyl)cyclohexane. Bistosylate (13.40 g, 0.028 mol) in 40 ml of warmed DMSO was added over 30 min to 6.25 g (10% excess) in 50 ml of DMSO rapidly stirred at 90°. The mixture was stirred at 80–90° for 1 hr, cooled, and poured onto 200 g of ice. The aqueous solution was extracted with four 80-ml portions of CCl_4 which was dried and concentrated. The residue was distilled to give 6.45 g of dibromide, bp 86–89° (0.45 mm), $[\alpha]^{25.9}D$ +57.1° (*c* 5.0, MeOH).

D. (+)-(*S,S*)-*trans*-1,2-Bis(isothiouroniummethyl)cyclohexane Dibromide. The above bisbromomethyl compound (6.45 g, 0.024 mol) in 25 ml of MeOH was added over 15 min to 4.85 g of recrystallized thiourea (0.063 mol) in 40 ml of stirred refluxing MeOH. The solution was gently refluxed 7 hr, cooled to room temperature, partially concentrated at reduced pressure, and then refrigerated. The crystalline deposit was recrystallized to constant melting point with EtOH–Et_2O, 9.81 g (91%), mp 234–235.5°, $[\alpha]^{26.0}D$ +57.97° (*c* 2.0 in MeOH). The ir was superimposable with that of racemic bis-isothiouronium salt prepared identically, mp 252°, lit.[74] 255°.

E. (+)-(*S,S*)-*trans*-1,2-Bis(thiolmethyl)cyclohexane. Bisisothiouronium salt (9.7 g, 0.023 mol) in 30 ml of 2 *N* NaOH solution was refluxed with rapid stirring for 90 min, cooled, acidified with 12 ml of 20% H_2SO_4, stirred 20 min, and then extracted with four 75-ml portions of Et_2O. The Et_2O was dried and concentrated and the residue was distilled, 2.30 g (57%), bp 147–148° (16.5 mm), lit.[74] bp 128–132° (10.5 mm), then redistilled, bp 73–75° (0.20 mm), $[\alpha]^{27.1}D$ +67.25° (*c* 2.55, MeOH).

F. (−)-(9*S*,10*S*)-*trans*-Hexahydro-2,3-benzodithiin ((−)-(*S,S*)-*trans*-2,3-Dithiadecalin). (+)-Dithiol (637 mg) in 50 ml of 1:1 MeOH–HOAc was added over 60 min to 1.35 g of $FeCl_3$ in 8 ml of MeOH and 3.5 ml of HOAc. The oxidation mixture was stirred 90 min, poured into 200 ml of ice and water, and then refrigerated overnight. The resulting white solid, 415 mg (64%), mp 77–83°, was recrystallized from MeOH to give glistening off-white needle clusters, mp 86–87° after drying *in vacuo*. The ir was superimposable with racemic disulfide prepared identically, mp 55.5–56.8°, lit.[74] mp 56.5–57°. Homogeneity was ascertained by glc of a heptane solution on 5-ft Porapak, SE-30, and Carbowax 20M, 15% on firebrick columns.

Anal. Calcd for $C_8H_{14}S_2$: C, 55.12; H, 8.10; S, 36.79. Found: C, 55.40; H, 8.13; S, 36.53.

Malformin A. Purified cyclopentapeptide was kindly provided by Professor Roy W. Curtis of Purdue University. Malformin A (59 mg), conformation I (MaI) was converted to malformin A, conformation III (MaIII) following Anzai and Curtis.[66e] The peptide was taken up in 20 ml of DMF warmed on a steam bath. The DMF was filtered to remove trace impurities and a slight amount of insoluble malformin A. The clear DMF solution was poured into 250 ml of warm 1% Na_2CO_3 and this mixture was warmed on the steam bath for 4 hr and then cooled. The precipitated malformin was filtered and dried *in vacuo* to give 8.1 mg of MaIII. Subsequent precipitation (18.0 mg) brought the total yield of MaIII to approximately the 50% reported by Curtis. Only the first crop of MaIII was used for spectroscopic investigation.

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