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Disulfide Bond Dihedral Angles from Raman Spectroscopy

(malformin A/stretching frequency/UV absorption/conformation)

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ABSTRACT **Raman spectra of several compounds containing the CS-SC moiety were obtained (in the solid phase) from 450–800 cm^{-1} to investigate the S-S and C-S stretching behavior. The S-S stretching frequency varied linearly with the CS-SC dihedral angle (obtained from either x-ray or neutron diffraction or ultraviolet absorption) for compounds whose CC-SS dihedral angles were not very different. The ratio of the intensities of the S-S and C-S stretching bands exhibited no recognizable correlation with either the CS-SC dihedral angle or the CSS bond angle, probably because this ratio is sensitive to the crystalline environment. The linear dependence of the S-S stretching frequency on dihedral angle leads to a dihedral angle for the plant hormone, malformin A, that is in excellent agreement with that estimated from the longest wavelength CS-SC ultraviolet absorption band.**

The disulfide bond is an important structural feature of many important biological molecules. The CS-SC moiety occurs in antibiotics such as gliotoxin, sporidesmin, and acetylaranotin in the form of an S-S bridged piperazinedione system in which the CS-SC dihedral angle is severely restricted by ring closure (1, 2). Hormones, such as oxytocin (3), vasopressin (4), and malformin (5), contain disulfide bonds (cystine) that can assume conformations with widely varying dihedral angle, depending upon ring size and other structural constraints. In globular proteins, such as ribonuclease, chymotrypsin, lysozyme, carboxypeptidase, etc., packing requirements may result in dihedral angles that vary substantially from the widely accepted value (6) of about 90° for L-cystine in aqueous solution. In chymotrypsin, for example, this angle varies from 87°–126° (7). It is, therefore, of considerable importance to have a method that can provide information about this dihedral angle both in solid state and in aqueous solution. Raman spectroscopy appears to be a suitable technique for this purpose, since the Raman spectra of compounds containing disulfide bonds usually show well-defined bands that arise from C-S and S-S stretching modes (8, 9), and these vibrations might be sensitive to the CS-SC dihedral angle.

Absorption spectra can also be used to obtain information about CS-SC dihedral angles, since the relationship between this dihedral angle and the longest-wavelength CS-SC absorption frequency in the ultraviolet is well established both theoretically (10) and experimentally (see references cited in ref. 10). However, this method for determining CS-SC dihedral angles is limited to simple systems in which the UV absorption spectrum is free from nondisulfide-related transitions from about 250 to 380 nm. Many systems (proteins for example, which contain aromatic residues) have nondisulfide chromophores that absorb in this region, and thus prevent study of the CS-SC absorption bands. On the other hand, as has been pointed out (8, 9), these compounds (including proteins) exhibit S-S and C-S stretching bands in a region of the Raman spectrum that is relatively free from other intense bands. Assignments of S-S and C-S stretching frequencies have been made for biological molecules as complex as lysozyme (8, 11), ribonuclease (12, 13), α-chymotrypsin (12), and other proteins (14–18).

The purpose of this paper is to use Raman spectroscopy to study various model compounds with widely different dihedral angles to try to find a correlation between the observed spectra and the conformational properties of the CS-SC group. UV absorption measurements are also made for those compounds for which suitable solvents are available, and the UV data are correlated with the Raman spectra.

MATERIALS AND METHODS

Materials. Dimethyl disulfide, D,L-6,8-thioctic acid, D,L-6,8-thioctic acid amide, L-cystine, and L-cystine·2HCl were obtained from commercial sources and used without further purification. L-Cystine·2HBr was prepared by action of aqueous HBr on L-cystine, and was recrystallized from tetrahydrofuran–water. Analysis: calculated for $C_6H_{14}N_2O_4S_2Br_2$: C, 17.92; H, 3.51; Br, 39.74. Found: C, 17.75; H, 3.66; Br, 39.53. Epi-di-thio sarcosine anhydride was a gift from Dr. Ivan Bernal of Brookhaven National Laboratory, and the 4-substituted 1,2-dithiolane (namely, 2-oxa-6,7-dithiaspiro-[3,4]octane) was synthesized by a modification of a procedure of Gunther and Salzman (19), as follows. 0.01 mol (1.55 g) of 3,3-bis(chloromethyl)oxetane [Borden Chem. Co., boiling point 61.5°C (3 mm)] in 30 ml of water was added to a hot (80°C) solution of sodium thiosulfate [0.02 mol (3.16 g) in 35 ml of water]. The reaction mixture was then refluxed for 2 hr after which time it was allowed to cool to room temperature and then placed in a refrigerator at about 10°C. A cold solution of sodium hydroxide (1.0 mol in 50 ml of water) was added to the intermediate dithiosulfate, which was not isolated at this stage. The reaction mixture became turbid upon addition of base and was allowed to stand at about 10°C for 20 hr. The crude product, which appeared as a light yellow precipitate, was dissolved in diethyl ether after the aqueous solution was decanted; the ether solution was dried over $MgSO_4$, filtered, and flash evaporated. The yellow disulfide (1.25 g) was then recrystallized twice from hexane to provide 0.93 g (63% yield) of the purified 2-oxa-6,7-dithiaspiro[3,4]octane as light yellow needles, melting point 52–53°C. Its nuclear magnetic resonance (NMR) spectrum showed only two single absorptions of equal intensity at 4.65 and 3.40 ppm downfield from tetramethylsilane. Analysis: Calculated for $C_5H_8S_2O$: C, 40.51; H, 5.44; S, 43.26. Found: C, 40.33; H, 5.20; S, 43.26.

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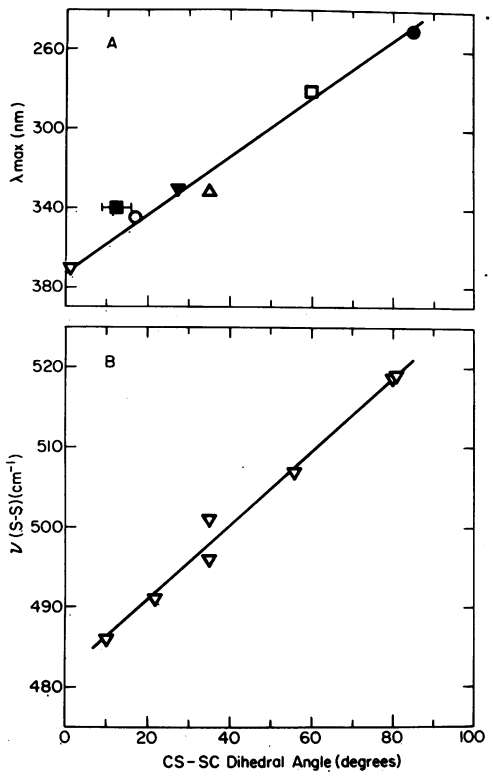


FIG. 1. (*A*) Dependence of longest wavelength UV absorption band on the CS-SC dihedral angle. (▽) 1α,5α-Epidithioandrostane-3α,17β-diol in alcohol (10, 34); (■) gliotoxin in alcohol (1); (○) acetylaranotin in alcohol (2, 35); (▼) 1,2-dithiolane-4-carboxylic acid in alcohol (24, 34); (△) thioctic acid in methanol (this work; 23); (□) 1,2-dithiane-3,6-dicarboxylic acid in alcohol (25, 36); (●) dimethyl disulfide in methanol (this work; 22). (*B*) Dependence of S-S stretching frequency on CS-SC dihedral angle. Data are from Table 1.

Spectroscopic Measurements. Raman spectra were recorded with a Spex 1401 spectrometer with the 488.0- and 514.5-nm exciting lines of a Coherent Radiation model 52G-A argon ion laser. Scattering was observed at 90° to the laser beam with a resolution of 2 cm^{-1}. Emission lines of the argon ion laser were used to calibrate the locations of the observed peaks in the spectra; they are accurate to ± 1 cm^{-1}. Spectra of those compounds that were yellow were also obtained with the 568.2-nm line of a Coherent Radiation model 52G-K krypton ion laser, and yielded identical results. Samples were prepared as described (20, 21). UV absorption spectra were recorded with a Cary model 14 spectrophotometer.

RESULTS

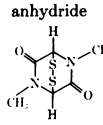
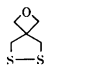
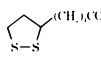
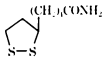
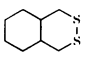
UV Absorption Data and Dihedral Angles. Information about the CS-SC dihedral angles of the compounds studied here was obtained from x-ray or neutron diffraction data. Where these were not available, UV absorption data were used to estimate this angle. Since all the compounds treated here (except dimethyl disulfide) contain rigid rings, we assume that the CS-SC dihedral angle is the same in solution and in solid phase. For dimethyl disulfide, which was examined as a liquid, its dihedral angle in the liquid was assumed to be the same as that in the gas phase [which was determined by microwave spectroscopy (22)]. In order to use UV absorption data to obtain CS-SC dihedral angles, we constructed the curve of Fig. 1*A*, from published UV absorption and x-ray and neutron diffraction data, for the compounds listed in the legend to Fig. 1*A;* for thioctic acid and dimethyl disulfide, the dihedral angles were obtained from x-ray (23) and microwave (22) data, respectively, and the UV absorption spectra were measured here. The diffraction and UV data of the compounds studied here are summarized in Table 1. From Fig. 1*A*, the weak band at about 380 nm (Table 1) for epi-di-thio sarcosine anhydride leads to a low value for the CS-SC dihedral angle, which is consistent with the value of 10° from x-ray data (Bernal, I., personal communication). From Fig. 1*A* and the absorption data of Table 1, the CS-SC dihedral angle of the 4-substituted 1,2-dithiolane studied here was estimated as 22°, which is a reasonable value since the dihedral angle of 1,2-dithiolane-4-carboxylic acid is 27° as determined from its x-ray structure (24). The value of 56° assigned here to the CS-SC dihedral angle of trans-2,3-dithiadecalin from its UV absorption spectrum (6) is also reasonable since the dihedral angle of 1,2-dithiolane-3,6-dicarboxylic acid is 60° (25). Since the influence of solvent on the UV absorption frequency is generally small, the variation in solvents among the compounds of Fig. 1*A* and Table 1 produces insignificant errors in the computed dihedral angles. The value of the CS-SC dihedral angle for L-cystine·2HCl was obtained from neutron diffraction data (Bernal, I., personal communication) and that for L-cystine·2HBr from x-ray data (26); however, the value reported (27) for L-cystine itself is incorrect (Bernal, I., personal communication), and this compound cannot be used to help establish the relation between the Raman spectrum and the CS-SC dihedral angle. These cystine dihedral angles pertain only to the crystalline compounds since these angles may change when the compounds are dissolved in water (28).

Raman Data. The Raman spectra in the S-S and C-S stretching regions, for the compounds studied here, are summarized in Table 1. We consider first the assignment of the S-S (and, in some cases, the C-S) stretching frequencies.

For epi-di-thio sarcosine anhydride, the 4-substituted 1,2-dithiolane, L-cystine, and trans-2,3-dithiadecalin, there is a single band in the 470- to 530-cm^{-1} region of the spectrum of each of these compounds; it may be identified with the S-S stretching mode with confidence (29). Since both hydrohalides of L-cystine have almost identical CS-SC dihedral angles and geometrical parameters about the S-S bond in the crystalline state, the common bands at 519 cm^{-1} and about 665 cm^{-1} are assigned to the S-S and C-S stretches, respectively, in these compounds. For L-cystine, we assign the 677 cm^{-1} band to the C-S stretch on the basis of data of Sheppard (29).

The S-S stretching regions in the spectra of thioctic acid and thioctic acid amide seem peculiar. Thioctic acid shows an intense peak at 511 cm^{-1} with a shoulder at about 501 cm^{-1} while thioctic acid amide has an intense peak at 496 cm^{-1} with a shoulder at 504 cm^{-1}. It is unlikely that the amidation of thioctic acid changes its CS-SC dihedral angle; this conclusion is supported by the identity of the 333-nm UV absorption bands for both of these compounds. Therefore, anticipating that a correlation might exist between the S-S stretching frequency and the CS-SC dihedral angle, the S-S stretch cannot be assigned on the basis of the band intensities, and we resort to an alternative procedure (described below) to assign these bands in thioctic acid and its amide.

TABLE 1. *Spectroscopic data for compounds containing disulfide bonds*

Compound	Raman data[a] ν cm^{-1}	Intensity[b]	Assignment	Reference	CS-SC dihedral angle (degrees)	λ_{max} (nm)	Bond length, Å (S-S)	Bond length, Å (C-S)	CSS bond angle (degrees)
Epi-di-thiosarcosine anhydride 	466	160		This work		Very weak band at ~380 (in dimethylsulfoxide)	2.07[c]	1.85[c]	99[c]
	486	660	S-S		10° (from x-ray)[c]				
	606	130							
	667	100							
	752	105							
	788	22							
4-substituted 1,2-dithiolane 	492	1430	S-S	This work	~22° (from UV)[d]	338 (in hexane)			
	689	100							
	715	11							
	731	215							
 D,L-6,8-Thioctic acid	456	67		This work	35° [x-ray (23)]	333 (in MeOH)	2.05(23)	1.83(23)	92.8(23)
	~501	sh	S-S					1.79(23)	95.5(23)
	511	370							
	559	63							
	634	93							
	682	100							
	In MeOH								
	504	380	S-S	This work	35° (assumed)				
	~526	sh							
	580	32							
	632	50							
	674	100							
 D,L-6,8-Thioctic acid amide	496	490	S-S	This work	~35° (assumed to be the same as thioctic acid)	333 (MeOH) (UV band identical to that of thioctic acid)	(probably same as above)		
	504	sh							
	533	66							
	585	120							
	664	58							
	675	100							
	708	92							
 Trans-2,3-dithiadecalin	506	400	S-S	(9)	~56° (from UV)	290 (in C_7H_{16}) (6) 292 (in CH_3CN) (6)			
	719	100							
	724	sh							
	747	40							
L-Cystine·2HCl	479	32		This work	81° (from neutron diffraction[c])		2.036[c]	1.822[c]	103.8[c]
	508	18							
	519	35	S-S						
	587	6							
	661	sh							
	665	100	C-S						
	752	37							
L-Cystine·2HBr	466	20		This work	81° [from x-ray (26)]		2.024(26)	1.862(26)	103.9(26)
	499	15							
	519	33	S-S						
	589	5							
	663	100	C-S						
L-Cystine	455	43		This work	The reported structure of this compound (27) is incorrect[c]				~104 (assumed)
	459	38							
	493	sh							
	499	1130	S-S						
	542	34							
	609	33							
	616	50							
	677	100	C-S						

[a] Compounds are pure solids unless specified otherwise.
[b] Measured as peak height, with 100 assigned to the prominent band in the C-S stretching region. *sh* = shoulder.
[c] Private communication from I. Bernal.
[d] The x-ray structure of this compound is being investigated by R. E. Hughes and M. Leonowicz.

The intensities of the S-S and C-S bands in the crystalline state vary widely, as indicated by the differences between cystine and its dihydrohalides. The intensity of the S-S band (relative to that of the C-S band) is large for cystine at 499 cm^{-1} but small for the dihydrohalides at 519 cm^{-1}. In fact, the assignment of the S-S mode might erroneously have been made to the comparably intense bands at 508 and 499 cm^{-1} (for the hydrochloride and hydrobromide, respectively). However, when all three cystine compounds are dissolved in 1 N HCl, the only bands observed in the 450- to 800-cm^{-1} region are the strong ones at 507 cm^{-1} and 666 cm^{-1}, attributed to S-S and C-S stretches, respectively. This illustrates that, in solution, the inherent strength of the S-S and C-S stretches in the Raman make these bands appear, whereas the other bands observed for these compounds in the solid (Table 1) disappear. This inherent strength is also illustrated by liquid alkyl disulfides (28). Thus, one may use the frequencies obtained from solution spectra to make assignments in solids whenever doubt exists as to which bands pertain to the S-S and C-S stretches. However, it should be kept in

mind that, while solution spectra do not contain the effects of crystalline fields present in the solid, conformational changes about the S-S and C-S bonds may be introduced (28) by dissolving the compound in a solvent (especially if the compound is not rigid), and both of these changes may affect the observed frequency of the S-S stretching band; this is an important point that will be considered below.

With all this in mind, the strong band at 504 cm^{-1} for thioctic acid in methanol (Table 1) can be attributed to the S-S stretch. This corresponds best with the band at 501 cm^{-1} for the solid phase spectrum, and hence this band is assigned as the S-S stretch. Poor solubility prevented us from obtaining the solution phase spectrum of thioctic acid amide; for lack of other experimental evidence, in this case only, we assign the S-S stretch to the more intense band at 496 cm^{-1}. The 4-substituted-1,2-dithiolane studied here, which also has a five-membered ring, shows a single band at 492 cm^{-1}, illustrating that the S-S stretch may well occur below 500 cm^{-1} for these five-membered rings. No explanation can be offered, at this time, to account for the differences between the spectra of thioctic acid and thioctic acid amide. However, some type of interaction between the sulfurs and the side chain is sterically possible, and might account for the observed changes in the Raman spectrum upon amidation.

It has been suggested (8) that the symmetric and asymmetric C-S stretching modes are degenerate for a CS-SC dihedral angle of 90° but that the degeneracy is removed as this angle departs from 90°; thus, the splitting of the C-S stretching bands might be a measure of the CS-SC dihedral angle. However, we find that no trend in the splitting of the C-S stretching bands in these compounds, if it exists, is obvious; further, by assuming that splitting does occur and then permuting the assignments of all neighboring bands in the C-S stretching region among symmetric and asymmetric modes, we find no correlation between such splittings and the CS-SC dihedral angle. One piece of experimental evidence argues against the assignment (8) of the 682-cm^{-1} band in thioctic acid to the asymmetric C-S stretching mode; while this is a B mode (30) and therefore should be depolarized (with a depolarization ratio ρ of about 0.75), the value of ρ was found here to be less than 0.2 for this band (674 cm^{-1}) when thioctic acid was dissolved in methanol.

Having considered the assignments of the bands, we see that the frequency of the S-S stretching mode appears to vary linearly with dihedral angle, as illustrated in Fig. 1*B*. However, as mentioned earlier, the frequency of the S-S stretching mode also depends on the conformation about the C-S bonds (i.e., on the CC-SS dihedral angles). In fact, the Raman spectrum of diethyl disulfide shows bands at 509 and 524 cm^{-1} that have been interpreted (28) as arising from the S-S stretching modes of *two* rotational isomers about the C-S bond, i.e., to two molecules whose CS-SC dihedral angles are the same, but whose CC-SS dihedral angles are different. Hence, it is important to keep in mind that variations in the CC-SS dihedral angle alone may affect the S-S stretching frequency to the extent of about 15 cm^{-1}.

An examination (with the aid of space-filling models) of the structures of the compounds used to construct Fig. 1*B* reveals that, with the exception of cystine·2HCl and cystine·2HBr, the CC-SS dihedral angles do not have the freedom to vary very much because of the restrictions of ring closure. In fact, in all of the compounds used to construct this figure, including the cystine dihydrohalides, the CC-SS dihedral angle corresponds to conformations in which the α-carbons are approximately gauche to the distal sulfur across the C-S bond. The 15 cm^{-1} difference between the S-S stretching frequencies for the rotational isomers of diethyl disulfide presumably arises from conformers in which these groups are *trans* across the C-S bond (28). The point to be made here is that Fig. 1*B* was constructed from compounds whose CS-SC dihedral angles varied, but whose stable conformation about the CC-SS bond did not depart appreciably from a gauche form. For this reason, we believe that the linear trend observed, in fact, arises from the change in the CS-SC dihedral angle. Unfortunately, since rotation about the C-S bond can itself have a large effect on the S-S stretching frequency, one cannot, at this stage, use Fig. 1*B* to estimate a CS-SC dihedral angle unambiguously from an S-S stretching frequency. Variations in both the CS-SC and CC-SS dihedral angles are probably responsible for the low value of 499 cm^{-1} for the S-S stretching frequency of cystine. Finally, we were not able to find any relationship between the CSS valence angle or CS-SC dihedral angle and the ratio of the intensities of the S-S and C-S stretching bands on the solid phase, as has been suggested (8, 14).

DISCUSSION

The assignments presented above, and the relation shown in Fig. 1*B*, may be used to interpret other published Raman spectra. For example, two similar 4-substituted 1,2-dithiolanes were examined in the solid phase, and (on the basis of their high intensities) the S-S modes were assigned to bands at 509 cm^{-1} (9). However, these two compounds also exhibited bands at 492 and 488 cm^{-1}, respectively. If this situation is similar to that described above for thioctic acid, then the S-S mode should be assigned to these lower frequencies rather than to 509 cm^{-1}. If so, the dihedral angles in these compounds would be expected to be in the vicinity of 20°, on the basis of Fig. 1*B*. This result is similar to that obtained here for the 4-substituted 1,2-dithiolane of Table 1, which has a single band in the S-S region at 492 cm^{-1}. While Raman or UV spectra in the liquid state could resolve this question, such experiments can not always be done because these compounds usually have limited solubility and tend to polymerize in solution.

A Raman spectrum, showing a single band at 487 cm^{-1}, has been reported for the plant hormone, malformin A (a cyclic pentapeptide with an S-S bond across the ring), in the solid phase (9). From Fig. 1*B*, we conclude that its CS-SC dihedral angle is about 12°. Since malformin A contains no aromatic residues that mask its S-S absorption region, its UV absorption maximum in concentrated HCl at about 365 nm (31) leads to a dihedral angle of about 6° (from Fig. 1*A*), in good agreement with the result from the Raman spectrum in this case. This result implies that the CS-SC and CC-SS dihedral angles in the solid phase are similar to those in concentrated HCl, and that the conformation about the C-S bond is gauche.

The reason for the behavior shown in Fig. 1*B* can be understood from a simple model. Using molecular orbital calculations, Boyd (10) has concluded that the S-S bond strength is greatest when the CS-SC dihedral angle is 90° and decreases as this angle departs from 90°. This behavior arises because the repulsion between the $3p_\pi$ lone-pair orbitals on

each sulfur is minimal at a dihedral angle of 90°, and increases as the dihedral angle departs from this value. From the limited available data in Table 1, the S-S bond lengthens slightly as the dihedral angle decreases (i.e., as the bond strength decreases). One would expect to find a smaller force constant, and hence a lower stretching frequency for the S-S bond, as the bond strength decreases, as observed in Fig. 1*B*. However, other effects (such as coupling between the CSS bending and S-S stretching modes) may be contributing to the behavior illustrated in Fig. 1*B*. It is not clear how Fig. 1*B* would appear for dihedral angles greater than 90°. Taking 0° as the cis conformation of the CS-SC moiety, dihedral angles of 0°–90° cannot be distinguished from those of 0° to −90° by Raman or UV measurements because of symmetry. The other half of the conformational range (which includes the *trans* conformation) remains to be investigated.

Finally, simple theoretical arguments may be cited to illustrate the danger of relying on intensities obtained from polycrystalline compounds to make band assignments. Because of the fixed and unique environment of a compound in a crystal, groups such as the CS-SC moiety will experience different environments with different relative intermolecular orientations from crystal to crystal. Since the intensity of a Raman band depends on the variation of the bond polarizability as the molecule vibrates, and this variation is affected differently by different environments in the crystal, the intensity will be affected. For example, L-cystine, L-cystine·2HCl, and L-cystine·2HBr all not only crystallize in different space groups, but also belong to different crystal systems, with different crystalline symmetries. In fact, Tobin (32) has cautioned that polarizabilities obtained from Raman experiments with crystals pertain to the crystal and not to the individual molecules. In general, only the frequencies (and not the intensities) can be relied on to reflect the Raman properties of the free molecule (33). Further, since the S-S stretch occurs at low frequency (about 500 cm^{-1}), this internal mode of the molecule may couple with an overtone of a lattice mode in the crystal. This could affect both the frequency and the intensity of a band such as an S-S stretch. All of these effects, which lead to differences in intensity between the solid and liquid phases, can be minimized by examining the spectra in solution where the environment of, say, the CS-SC moiety is averaged over all molecular orientations, thereby eliminating intermolecular crystal effects. Thus, wherever possible, assignments of S-S and C-S bands should be made by examining the spectrum not only in the solid but also in the liquid phase.

It appears that laser Raman spectroscopy is potentially a very useful tool for determining the conformation of disulfide bonds in polypeptides and proteins.

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