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# Conformational Preferences of the Bridging Groups of Cyclo-L-cystine and the Active Fragments of Epipolythiodiketopiperazine Antibiotics[1a]

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**Abstract:** Simple quantum chemical calculations of the extended Hückel (EH) type have been performed for model systems to elucidate the conformational preferences of the bridging groups of cyclo-L-cystine (3,6-epi-$CH_2S_2CH_2$-2,5-diketopiperazine) and the active fragments of 3,6-epi(dithio- and tetrathio-)2,5-diketopiperazine antibiotics. The conformational preferences inferred from the EH calculations are the same as those deduced from a classical analysis of the van der Waals interactions between the bridging atoms and the amide carbon and nitrogen atoms. They agree with the results of x-ray analyses of these compounds. Experimental and theoretical results indicate that hydrogen bonding to the carbonyl oxygens does not alter the conformational preference of any of these systems. A comparison of EH and ab initio results reveals that the EH model is inadequate for describing the effect of C=O···H—O hydrogen bonding on the peptide moiety.

## Introduction

The 2,5-diketopiperazine (DKP) ring bridged by a disulfide group across the 3,6 positions is now well established as the active fragment of a group of biologically interesting fungal metabolites that have toxic, antibacterial, antiviral, and cytostatic properties.[2]

Five of these metabolites have now been subjected to detailed structural analysis by x-ray crystallographic methods. They are sporidesmin,[3] gliotoxin,[4] antibiotic LL-S88$\alpha$, which was shown to be acetylaranotin,[5,6] chaetocin,[7] and antibiotic A26771A.[8] In all of these compounds the helicity of the disulfide bond is such that each sulfur atom is closer to the adjacent carbonyl carbon atom (structure I, Figure 1) rather than to the adjacent nitrogen atom (IV, Figure 1). The CSSC dihedral angles (DAs) vary from 8 (chaetocin) to 18° (acetylaranotin), their signs being determined by the chirality of the asymmetric centers.

Molecular orbital calculations on compounds with amide groups[9-13] consistently show that amide carbon and nitrogen atoms are electron-deficient and electron-rich centers, respectively, provided that the calculation takes the "$\pi$" and "$\sigma$" electrons into account on an equal footing.[9] Experimental[14,15] and theoretical[16-21] work has established that the ground states of hydrogen disulfide, HSSH, and dimethyl disulfide, $H_3CSSCH_3$, have a clear preference for a twisted geometry in which the DA is ~90°. Hence it has been suggested that the observed conformational preference of the disulfide linkage in 3,6-epidithio-2,5-DKP systems is due to intramolecular electrostatic attraction between the sulfur lone-pair electrons and the positively charged amide carbon atoms[8,18,20] and to repulsion between the sulfur and nitrogen lone pairs.[20]

All naturally occurring, biologically active metabolites of this type were thought to contain epidithio bridges until Taylor and his coworkers isolated first an epitrithio derivative and then an epitetrathio derivative, sporidesmin G, from natural sources.[22,23] On the basis of the above considerations one would expect the tetrasulfide bridge in an epitetrathio system to be skewed in such a way as to bring the inner sulfur atoms closer to the carbonyl carbons of the DKP ring (VI) than to the nitrogen atoms (V). The structures of three such systems have now been determined by x-ray crystallography. They include a synthetic system, 3,6-epitetrathio-$N^1$,$N^4$-dimethyl-2,5-DKP,[24] sporidesmin G etherate,[25] and a derivative of hyalodendrin.[26] In each case the tetrasulfide bridge is found to be twisted so that its inner sulfurs are closer to the nitrogens and its outer sulfurs are closer to the carbonyl carbons. It has been suggested that the nitrogen atoms in the tetrathio compounds are positively charged and that the observed stereochemistry is therefore a consequence of electrostatic attraction between the sulfur and nitrogen atoms.[25] This explanation rests, however, on the invalid assumption that the $\pi$-electron approximation holds for the amide group.[9]

Another bridged 2,5-DKP, cyclo-L-cystine, has been studied by a variety of spectroscopic techniques because of its importance as an experimental model for testing the theory of the chiroptical properties of disulfides.[27-29] In cyclocystine, the 2,5-DKP ring is bridged by a $CH_2S_2CH_2$ group across the 3,6 positions. As in the case of the dithio and tetrathio derivatives, one can try to predict the more likely helicity of the bridge from a consideration of the probable transannular electrostatic and nonbonded overlap interactions. Again a conformation (VIII) in which the sulfur atoms are proximate to the carbonyl carbon atoms is expected to be preferred over one (VII) in which the

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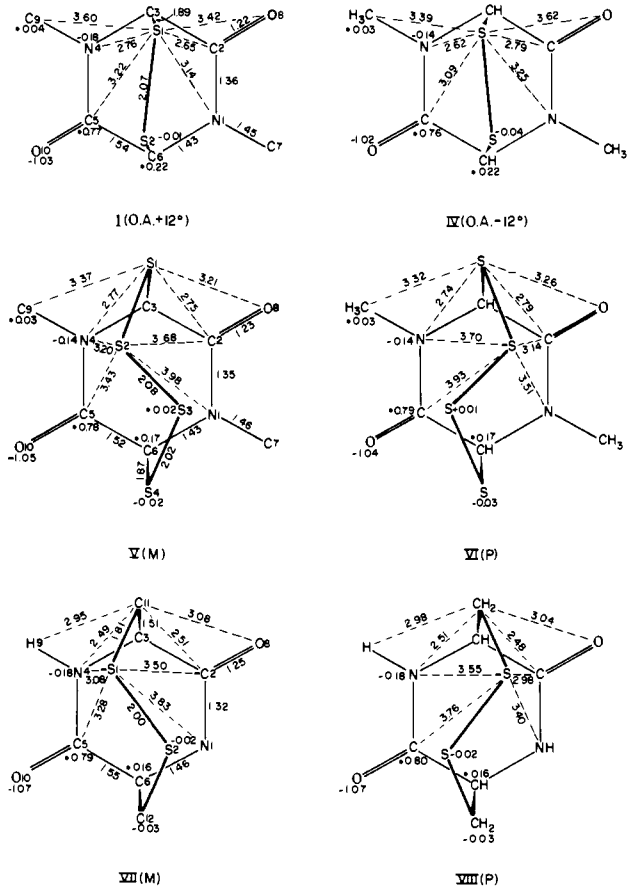

Figure 1. The model dithio, tetrathio, and $CH_2S_2CH_2$ systems and the numbering scheme. Unsigned quantities are bond lengths and transannular distances in angstroms. Signed quantities are net atomic charges in units of |e|. The hydrogen atoms have been omitted from the majority of the structures on the left for the sake of clarity.

sulfur atoms are proximate to the nitrogen atoms. The spectroscopic evidence points to the conclusion that VIII is the dominant conformer in polar and hydrogen-bonding solvents.[27-29] Furthermore, it was discovered that the chiroptical behavior of cyclocystine is time dependent upon dissolution.[29] This unexpected complication was attributed to a change in conformation on going from the crystal to solution. The observed time dependence led to the tentative conclusion that the conformational change involves a transition from VIII to a mixture of VII and VIII.[29]

Although these three observations are consistent with the premise that VIII is the thermodynamically more stable conformer, it would be premature to conclude that the premise is true because the disulfide bond of cyclocystine in crystalline cyclocystineacetic acid has been found to have the helicity shown in VII.[30] However, there is a hydrogen bond between the hydroxyl group of the acetic acid molecule and the amide oxygen and it has been implied that this bond may be responsible for the observed helicity.[29] If this were so, one could reasonably expect the same type of hydrogen bonding to influence the helicity of the bridging groups of the dithio and tetrathio derivatives of 2,5-DKP in the crystalline state. However, as shown in Table I, the helicity is the same irrespective of whether the amide carbonyl group is hydrogen bonded or not.

In these circumstances conformational energy calculations on representative model systems are clearly warranted. One may adopt either the classical approach based on empirical potential functions or the quantum chemical approach. Although the classical approach has already been used to study a related system,[31] we felt that we could obtain insights into the conformational preferences of the model systems more easily by using a standard quantum chemical procedure.

Table I. Hydrogen-Bonded Carbonyl Groups Found in the Crystals of 3,6-Epi(dithio and tetrathio) Derivatives of 2,5-DKP[a]

| Compound | Ref | Hydrogen bonds |
|---|---|---|
| | $-S_2-$ | |
| Antibiotic LL-S88$\alpha$ | 5, 6 | None |
| Sporidesmin | 3 | intra C=O···H—O |
| Gliotoxin | 4 | intra C=O···H—O<br>inter C=O···H—O |
| Chaetocin | 7 | intra C=O···H—N<br>inter C=O···H—N |
| Antibiotic A26771A | 8 | intra } not fully<br>inter } described |
| | $-S_4-$ | |
| 3,6-Epitetrathio-$N^1$,$N^4$-dimethyl-2,5-DKP | 24 | None |
| Sporidesmin G etherate | 25 | None |
| Derivative of hyalodendrin | 26 | inter C=O···H—O<br>inter C=O···H—O |

[a] intra = intramolecular, inter = intermolecular.

## Experimental Section

Since the size of the model systems (Figure 1) rules out an ab initio study at the present time, we turned to more approximate methods. The standard approximate methods are semiempirical, all-valence electron methods and can be classified as one-electron or two-electron theories and as full-overlap or neglect of differential overlap (NDO) methods. Most are based on Hartree-Fock (HF) molecular orbital (MO) theory. In the one-electron theories such as the extended Hückel (EH) theory[32] all electron-electron interaction integrals are neglected, whereas in the two-electron theories such as the complete (C) NDO/2,[33] intermediate (I) NDO,[34] and modified (M) INDO/2,[35] theories only the three- and four-center electron-electron interactions are neglected entirely.

These methods have been used extensively in theoretical conformational analysis over the past decade. Success has been mixed, but many of their reported failures, and perhaps some of their successes, too, bear testimony more to the unreliability of using experimental or standard geometrical parameters than to the intrinsic unreliability of the methods themselves.[36] At first, they appeared to fail without warning,[37] but now enough results have been reported to discern patterns of success and failure which can be used to select the most appropriate method for a particular problem. Thus NDO methods generally tend to favor more crowded and more highly connected structures.[38,39] Schemes based on the CNDO and INDO approximations suffer from the intrinsic defect that they underestimate repulsions between lone pairs of electrons.[35] The CNDO/2 and INDO methods[33,34] sometimes underestimate both lone-pair and nonbonded overlap repulsions to an extent that leads to unrealistic results.[36d,40-42] The latter two deficiencies are sufficient to preclude these methods from further consideration here.

The only standard quantum chemical method which is not based on the HF model is the two-electron perturbative configuration interaction using localized orbitals (PCILO) method.[43] Since it is only applied in conjunction with the approximations and parameterization of the CNDO/2 method, it likely shares some of the deficiencies of the CNDO/2 and INDO methods. Comparison of potential energy curves calculated for acetylcholine[36b-d,40,44a] and for the neutral and protonated forms of phenethylamine[44b] by classical and quantum chemical methods indicates that the CNDO/2, INDO, and PCILO methods all underestimate nonbonded repulsions to a similar and unacceptable degree. The PCILO method also gives the least satisfactory description of the conformation of dimethyl disulfide of all of the semiempirical methods that have been applied to this molecule to date.[17,19,21] We have therefore rejected the PCILO method, too, for the present problem.

Unlike the CNDO/2, INDO, and PCILO methods, the EH method, a full-overlap, one-electron method, appears to be able to cope with nonbonded repulsions in a qualitatively correct fashion.[42,44-47] Also, despite its many well-known deficiencies and limitations,

**Table II.** Parameters Used in the Extended Hückel Calculations[a]

| Element | Exponent | s (VSIP), eV | p (VSIP), eV |
|---|---|---|---|
| H | 1.2 | 13.6 | |
| C | 1.625 | 21.4 | 11.4 |
| N | 1.95 | 26.0 | 13.4 |
| O | 2.275 | 29.0 | 14.5 |
| S | 1.827 | 20.0 | 13.3 |

[a] Wolfsberg-Helmholz proportionality constant, $K = 2.0$.

Hoffmann and his coworkers have demonstrated many times that EH theory is capable of producing reliable insights into chemical bonding and reactions. In addition, Boyd[18-20] has carried out an exhaustive comparison of the results of EH and ab initio calculations for hydrogen disulfide and established that the changes in MO energies, total energy, and charge distribution that are predicted by these methods to accompany torsion of the disulfide bond are qualitatively similar. The EH method also describes the qualitative features of the net charge distribution in the peptide moiety correctly.[9-13]

We have therefore elected to investigate the conformational preferences of the model systems of interest here with the EH method. Solution of the characteristic EH eigenproblem yields the MOs and their energies, $\epsilon_i$. We took the total electronic energy to be $2\Sigma_i\epsilon_i$ where the sum is over all (doubly) occupied orbitals. The electronic wave functions were subjected to a Mulliken population analysis[48] which yields gross atomic populations and overlap populations.

The results of both EH and ab initio calculations indicate that the main features of the ground state of hydrogen disulfide can be described satisfactorily without including S 3d atomic orbitals (AOs) in the basis.[18,21] Consequently, the AO basis was limited in the present work to a minimal, isotropic, valence basis of Slater-type s and p orbitals for each nonhydrogenic element. The AO exponents, valence-state ionization potentials (VSIPs), and Wolfsberg-Helmholz proportionality constant that were used are shown in Table II. We used a modified version of Program No. 64, "EXTHUC", distributed by the Quantum Chemistry Program Exchange, Indiana University, Bloomington, Ind. It does not seem to have been noted very often that this program may fail to give reliable results if the orbital exponents adopted for two different elements are too similar. This observation helped guide our choice of orbital exponent for sulfur; otherwise, our parameter choices are those advocated by Boyd.[11]

The geometry of the model dithio compound, 3,6-epidithio-$N^1$,$N^4$-dimethyl-2,5-DKP, was derived from the geometry of antibiotic A26771A in the following way. First, the benzyl and $CH_2OH$ groups which are attached to the apical carbons in the antibiotic were replaced with hydrogen atoms. Second, the atomic coordinates reported by Michel et al.[8] for the remaining fragment of the antibiotic were averaged in such a way that it took on $C_2$ symmetry, the twofold axis passing through the center of the disulfide bond. Finally, the coordinates of the hydrogen atoms of the methyl groups were adjusted to preserve $C_2$ symmetry and to give CH bond lengths of 1.10 Å and tetrahedral NCH bond angles.

Holding the geometry of the $N^1$,$N^4$-dimethyl-2,5-DKP ring fixed, we performed calculations for four values of the CSSC DA: +12° (I), +6° (II), 0° (III), and −12° (IV).[49] To obtain models II and III from model I, the sulfur atoms were moved at right angles to the line joining $C_3$ and $C_6$. Consequently, the $S_1$–$C_3$ and $S_2$–$C_6$ bond lengths remained constant, while the $S_1$–$S_2$ bond length decreased to 2.05 Å in II and 2.04 Å in III. The $N^1$,$N^4$-dimethyl-2,5-DKP ring has the form of a slightly twisted boat. The apical carbons lie 0.5 Å above the mean plane defined by the other four atoms of the DKP ring. The disulfide group lies 2.38 Å above this plane.

3,6-Epitetrathio-$N^1$,$N^4$-dimethyl-2,5-DKP was chosen as the model tetrathio compound. This molecule has $C_2$ symmetry. We performed calculations on two geometries. One was the geometry (V) determined by Davis and Bernal[24] in which the inner sulfur atoms $S_2$ and $S_3$ are displaced toward the nitrogen atoms of the DKP ring and the $S_1$–$S_2$ and $S_2$–$S_3$ DAs are 68.6 and −105.3°, respectively. The other was the geometry (VI) in which the helicity of the tetrathio bridge is reversed so that the inner sulfur atoms are displaced toward the carbonyl carbons. In this compound the apical carbons lie only 0.24 Å above the mean plane of the other four atoms of the DKP ring, which again has a distorted boat form. The inner sulfurs lie 3.12 Å above this plane. CH bond lengths and NCH bond angles were fixed in the same way as for the model dithio compound.

The geometry of the model $CH_2S_2CH_2$ compound, cyclocystine, was derived from the structure reported[30] for cyclocystineacetic acid by averaging the structure of the cyclocystine moiety in such a way that it took on $C_2$ symmetry. In the resultant geometry (VII), the sulfur atoms are proximate to the nitrogen atoms and the S–S DA is −91.5°. We performed calculations on this geometry and on the geometry VIII in which the helicity of the $CH_2S_2CH_2$ bridge is reversed. The apical carbon atoms lie 0.32 Å above the mean plane of the other four atoms of the DKP ring. The disulfide group lies 2.97 Å above this plane.

The effect of methyl substitution at the nitrogens on the conformational preference of cyclocystine was calculated in a similar way (models IX and X). We also examined the effect of hydrogen bonding of the type found in crystalline cyclocystineacetic acid.[30] To do this, we oriented a water molecule relative to the averaged cyclocystine structure so that it mimicked the acetic acid molecule in the complex. However, EH theory has enjoyed mixed success in characterizing hydrogen-bonded systems.[39,46,50] It is therefore of interest that ab initio results were reported recently[51] for a hydrogen bonded formamide-water system labeled $T'_{00}$ in which the free water hydrogen is trans to the carbonyl bond as in the model hydrogen-bonded cyclocystine. These results afford an excellent opportunity to evaluate the reliability of the EH method for assessing the effect of hydrogen bonding on the peptide linkage. Accordingly, we also performed EH calculations on the $T'_{00}$ system and its isolated components. The EH calculations were carried out for the partially optimized geometries that were obtained in the ab initio calculations.[51]

## Results and Discussion

The net atomic charges calculated for a number of models are shown in Figure 1. The charges on the amide atoms are consistent with the results of previous calculations[9-13] on compounds with amide groups. The exaggerated charge separations are characteristic of EH results for systems with heteroatoms. Also, it is possible that the sulfur atoms are predicted to be slightly too electropositive because S 3d AOs were excluded from the basis. However, the sulfur charges compare favorably with those obtained from earlier calculations[11,19-21] in which S 3d AOs were included in the basis. In particular, Boyd[19] obtained a net atomic charge of $-0.04|e|$ for the sulfur atoms of dimethyl disulfide when the CSSC dihedral angle was 90°.

Overlap populations (OPs) for the bonded atoms and total energies (TEs) are given in Table III. There is a reasonably good inverse correlation between OP (Table III) and bond length (Figure 1). When the present results are compares with the results of ab initio calculations for amides,[13] one finds, as others[20,47] have found, that the EH method overestimated OPs between bonded atoms. The semiempirical method may also fail to order the OPs for bonds between different elements correctly. Thus the OP for the $N_1$–$C_2$ bond is exaggerated so much that it even exceeds that for the $C_2$–$O_8$ bond. Fortunately, only the conformational dependence of these quantities is of interest here. The OPs between the sulfur atoms and the amide carbon and nitrogen atoms of the model dithio compound are plotted in Figure 2 as a function of the CSSC DA. Those between the bridging atoms and other centers in the model tetrathio and $CH_2S_2CH_2$ compounds are listed in Table IV.

In a one-electron theory such as EH theory, covalent bond energies can be identified with the quantities Mulliken[52] calls "overlap energies". The overlap energy, $\Omega(r_k s_l)$, for the two orbitals r and s of atoms k and l, respectively, is given approximately by

$$\Omega(r_k,s_l) \approx A_\tau \bar{I}_{rs} OP(r_k,s_l)$$

where $A_\tau$ is an empirical constant which depends on the nature of the orbitals r and s and $\bar{I}_{rs}$ is the negative of their mean VSIP. To obtain a rough estimate of the significance of the nonbonded OPs shown in Figure 2 and Table IV, we shall set

Table III. Overlap Populations (OPs) for Bonded Atoms and Total Energies (TEs) for Conformers of the Model $S_2$, $S_4$, and $C_2S_2$ Bridged DKPs

| | System | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *a* | | *b* | | *c* | | *d* | |
| Bond | I | IV | V | VI | VII | VIII | IX | X |
| $N_1$-$C_2$ | 1.0211 | 1.0361 | 1.0481 | 1.0534 | 1.0751 | 1.0757 | 1.0930 | 1.0934 |
| $C_2$-$C_3$ | 0.8091 | 0.8167 | 0.8161 | 0.8116 | 0.8032 | 0.7956 | 0.8018 | 0.7944 |
| $C_3$-$N_4$ | 0.7541 | 0.7414 | 0.7340 | 0.7286 | 0.7169 | 0.7125 | 0.7283 | 0.7242 |
| $N_1$-$C_7$ | 0.7129 | 0.7162 | 0.7121 | 0.7225 | | | 0.7100 | 0.7104 |
| $N_1$-$H_7$ | | | | | 0.7278 | 0.7281 | | |
| $C_2$-$O_8$ | 0.9478 | 0.9486 | 0.9350 | 0.9380 | 0.9109 | 0.9131 | 0.9063 | 0.9088 |
| $C_3$-$S_1$ | 0.6581 | 0.6685 | 0.7155 | 0.7412 | | | | |
| $C_3$-$C_{11}$ | | | | | 0.7433 | 0.7477 | 0.7442 | 0.7485 |
| $S_1$-$S_2$ | 0.6854 | 0.6818 | 0.6955 | 0.6885 | 0.7039 | 0.7015 | 0.7039 | 0.7016 |
| $C_{11}$-$S_1$ | | | | | 0.7518 | 0.7449 | 0.7517 | 0.7449 |
| $S_2$-$S_3$ | | | 0.6596 | 0.6574 | | | | |
| −TE, eV | 1243.65 | 1243.16 | 1442.80 | 1442.85 | 1243.12 | 1242.89 | 1468.83 | 1468.55 |

[a] 3,6-Epidithio-$N^1$,$N^4$-dimethyl-2,5-DKP (conformers I and IV). [b] 3,6-Epitetrathio-$N^1$,$N^4$-dimethyl-2,5-DKP (conformers V and VI). [c] 3,6-Epi-$CH_2S_2CH_2$-2,5-DKP (conformers VII and VIII). [d] 3,6-Epi-$CH_2S_2CH_2$-$N^1$,$N^4$-dimethyl-2,5-DKP (conformers IX and X).

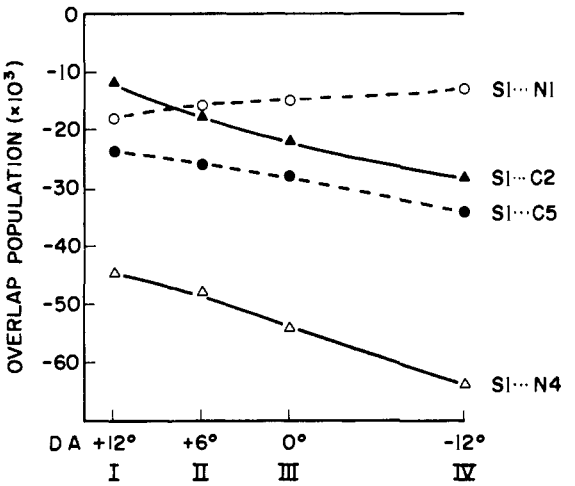


Figure 2. Overlap populations for the nonbonded interactions between the sulfur atoms and the amide carbon and nitrogen atoms of the model dithio compound plotted as a function of the CSSC dihedral angle.

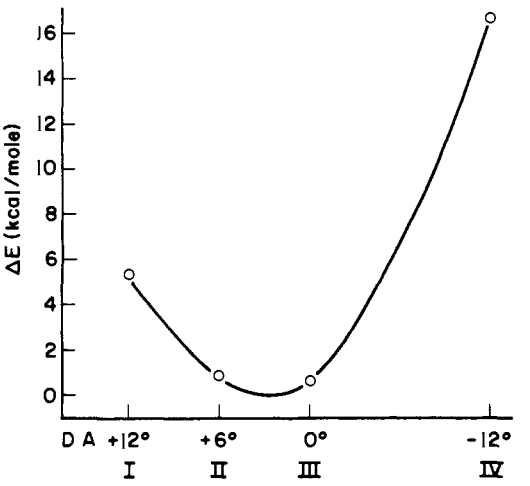


Figure 3. Total energy (in kilocalories/mole) of the model dithio compound relative to its minimum value plotted as a function of the CSSC dihedral angle.

Table IV. Overlap Populations (×10³) between the Bridging Atoms and Other Centers in the Model Tetrathio and $CH_2SSCH_2$ Compounds

| 3,6-Epitetrathio-$N^1$,$N^4$-dimethyl-2,5-DKP | | |
|---|---|---|
| | V, $S_2$ closer to $N_4$ | VI, $S_2$ closer to $C_2$ |
| $S_1$···$N_4$ | −45 | −53 |
| $S_1$···$C_2$ | −42 | −42 |
| $S_1$···$C_9$ | −6 | −8 |
| $S_1$···$O_8$ | −5 | −4 |
| $S_2$···$N_4$ | −9 | −1 |
| $S_2$···$C_2$ | −5 | −6 |
| $S_2$···$C_5$ | 6 | 0 |
| $S_2$···$N_1$ | 0 | −2 |
| 3,6-Epi-$CH_2S_2CH_2$-2,5-DKP | | |
| | VII, $S_1$ closer to $N_4$ | VIII, $S_1$ closer to $C_2$ |
| $C_{11}$···$N_4$ | −47 | −44 |
| $C_{11}$···$C_2$ | −44 | −45 |
| $C_{11}$···$H_9$ | −4 | −4 |
| $C_{11}$···$O_8$ | −4 | −4 |
| $S_1$ ···$N_4$ | −14 | −2 |
| $S_1$ ···$C_2$ | −7 | −5 |
| $S_1$ ···$C_5$ | 9 | 0 |
| $S_1$ ···$N_1$ | 0 | −4 |

$A_\tau = 1$ and consider, say, the N 2p and S 3p AOs. It then follows that

$$\Omega \text{ (N 2p, S 3p)} \simeq -310 \text{ OP (N 2p, S 3p) kcal/mol}$$

Hence OPs with magnitudes as small as $\sim 5 \times 10^{-3}$ are chemically significant. Changes in OP of this order of magnitude have recently been used as a measure of reactivity.[53]

The total electronic energy of the model dithio compound is plotted in Figure 3 as a function of the CSSC DA. The model compound is predicted to have a strong preference for a geometry in which the sulfurs are displaced toward the carbonyl carbons. The minimum energy geometry occurs at a DA of ca. +3°, an angle somewhat smaller in magnitude than the smallest one so far observed. However, predicting the exact position of the minimum is less important in rigid geometry calculations than correctly predicting the overall conformational preference of the molecule. Presumably, the calculations are then adequate for explaining that preference.

It is apparent from Figures 1 and 2 that the electrostatic and nonbonded covalent interactions between the sulfur and the amide carbon and nitrogen atoms favor the observed conformation. The $S_1$···$C_9$ nonbonded covalent interaction also favors the observed conformation (OPs: I, $-3 \times 10^{-3}$; IV, $-8 \times 10^{-3}$). The $S_1$···$O_8$ interaction does not, but it is less sensitive to the DA (OPs: I, $-3 \times 10^{-3}$; IV, $-1 \times 10^{-3}$). Small differences in the OPs between bonded atoms (Table III) indicate

that slightly different geometries would be obtained if the molecule were allowed to relax while keeping the DA fixed. They favor the geometry in which the sulfur atoms are displaced toward the nitrogens, but the combined effect of the nonbonded electrostatic and covalent interaction between the bridge and the 2,5-DKP ring is sufficient to outweigh them and to establish a strong overall preference for the observed conformation.

In contrast to the model dithio compound, the model tetrathio compound is not predicted to have a strong conformational preference. The alternative conformer is calculated to be slightly more stable than the observed one, but the difference in TEs is only 1 kcal/mol. If the inner sulfurs carry a slight positive charge, as the present calculations suggest (Figure 1), then all of the electrostatic interactions between the bridging atoms and the amide carbons and nitrogens favor the observed conformation. The nonbonded covalent interactions between the outer sulfurs and the amide centers (Table IV) clearly favor the observed conformation. As expected from the larger distances involved, those between the inner sulfurs and the amide atoms are small. Neither they nor the bonded OPs (Table III) clearly favor one conformer over the other.

In agreement with the calculations, the outer sulfurs in the three x-ray analyzed tetrathio compounds are all skewed slightly toward the amide carbons rather than toward the amide nitrogens. This situation resembles that in the dithio compounds. Since the interactions between the inner sulfurs and the amide centers are weak, it seems likely that, as the outer sulfurs are pulled toward the amide carbons, the inner sulfurs' preference to take up M helicity is due less to their interactions with the amide centers than to the fact that the S–S bonds are then able to attain larger DAs and nearly equal S–S–S bond angles (103, 105°) and hence a more stable staggered conformation. Nevertheless, on the basis of our calculations, one would also expect conformer VI in which the outer sulfurs are skewed slightly toward the amide nitrogens and the inner sulfurs are nearer to the amide carbons to be represented in nature.

The helicity of cyclocystine is predicted to be the same as that found in crystalline cyclocystineacetic acid.[30] However, no explanation for this preference is to be found in the molecular charge distribution (Figure 1), in the nonbonded covalent interactions between the bridging atoms and other atoms (Table IV), or in the skewing of the bridging carbon atoms. The electrostatic interactions favor the alternative conformer and the combined effect of the changes in the covalent ones is negligible to a good approximation. As in the case of the model dithio and tetrathio compounds, differences between conformers in the nonbonded covalent interactions across the DKP ring are also negligible. In the EH model, the small but significant differences between conformers in the bonded OPs (Table III) are responsible for the observed preference. The relative insensitivity of the $N_1$–$C_2$ and $C_3$–$C_{11}$ bonds to the helicity of the $CH_2S_2CH_2$ bridge is an important factor contributing to the extra stability of VII.

The effect of methylation at the nitrogens of cyclocystine is to increase its preference slightly for the geometry in which the sulfurs are nearer to the nitrogens. Except for small positive and negative shifts in the charges carried by the nitrogen ($-0.18 \rightarrow -0.11|e|$) and carbonyl carbon ($0.79 \rightarrow 0.76|e|$) atoms, respectively, changes in the molecular charge distribution are negligible. According to the bonded OPs (Table III), methylation strengthens all of the C–N bonds in the DKP ring, slightly weakens the carbonyl bonds, and has a negligible effect on the rest of the molecule. These trends, which are somewhat similar to those apparent in ab initio results for formamide and *N*-methylformamide,[13] will slightly alter the disposition of the bridge relative to the DKP ring and thus have an indirect effect on conformational stability. This is unlikely to be important

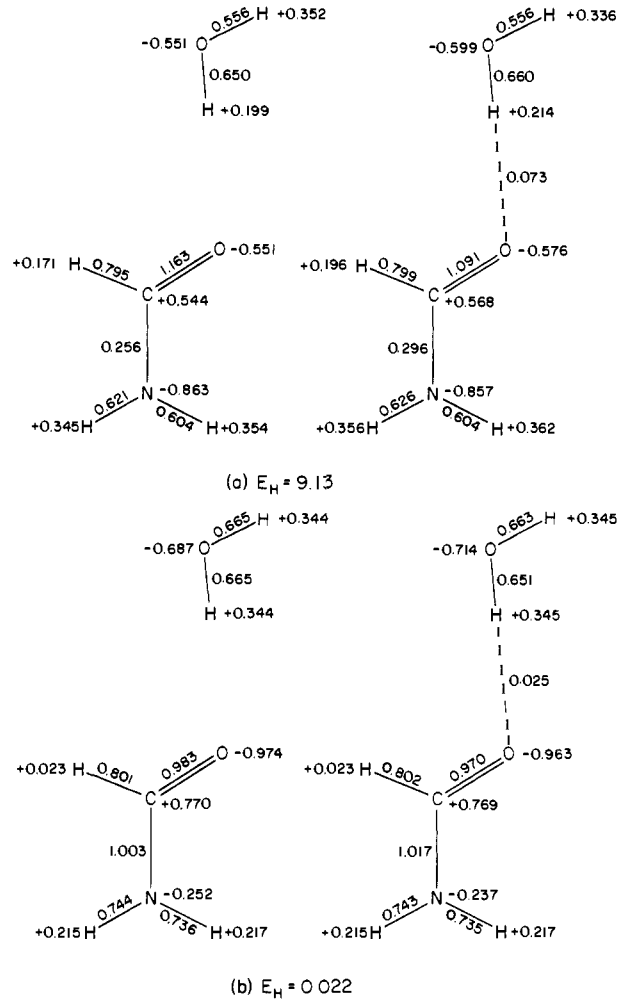


**Figure 4.** Ab initio (a) and EH (b) net charges and bonded OPs for the $T'_{00}$ complex (RHS) and its isolated components (LHS). $E_H$ is the hydrogen bond energy in kilocalories/mole.

because the bridge–DKP ring interactions are not responsible for the overall conformational preference of the cyclocystine molecule.

Similarly, hydrogen bonding has both a direct and an indirect effect on conformational stability. It perturbs the electronic structure of the molecule directly and the bridge–DKP ring interactions indirectly through geometry changes. The empirical data presented in Table I indicate that the conformational preferences of the dithio and tetrathio derivatives of 2,5-DKP are not altered by hydrogen bonding to the carbonyl oxygens. In view of this observation and the results of the calculations for cyclocystine, it seems clear that the helicity observed for cyclocystine in crystalline cyclocystineacetic acid is not due to the hydrogen bond to the carbonyl oxygen. The results of ab initio calculations on C=O···H—O bonded formamide–water systems[51] and those of x-ray crystal structure studies on 3,6-pyridazinediones[51] support this conclusion. They indicate that the electronic structures of the proton acceptors are not strongly perturbed by the type of hydrogen bond under consideration. Changes in the C=O and C—N bond lengths are small (<0.01 Å). However, if the nitrogen atoms of the dipeptide ring were protonated, then the order of stability of the conformers might be different in these systems.

The net charges, bonded OPs, and hydrogen bond energies, $E_H$, that were obtained for the $T'_{00}$ complex and its isolated components from ab initio calculations[51] and EH calculations (this work) are compared in Figure 4. The ab initio calculations

Table V. Shortest van der Waals Type Transannular Distances ($d$) in Models I-VIII of Epithio-DKP Derivatives and the Degree of Shortening ($d_w - d$) from the Sums of van der Waals Radii ($d_w$)

| | $r_w$,[a] Å | | $d_w$, Å |
|---|---|---|---|
| C | 1.53[b] | S···C | 3.18 Å |
| N | 1.46[b] | S···N | 3.11 |
| O | 1.42[b] | S···O | 3.07 |
| S | 1.65[c] | C···O | 2.95 |

| | | $d$ | $d_w - d$ |
|---|---|---|---|
| Model I ($-S_2-$) | $S_1\cdots N_1$ | 3.14 | |
| Model IV ($-S_2-$) | $S_1\cdots C_5$ | 3.09 | 0.09 |
| Model V ($-S_4-$) | $S_2\cdots N_4$ | 3.20 | |
| Model VI ($-S_4-$) | $S_2\cdots C_2$ | 3.14 | 0.04 |
| Model VII ($-C_2S_2-$) | $S_1\cdots N_4$ | 3.08 | 0.03 |
| Model VIII ($-C_2S_2-$) | $S_1\cdots C_2$ | 2.98 | 0.20 |

[a] $r_w$, van der Waals radius. [b] A. Bondi, *J. Phys. Chem.*, **68**, 441 (1964). [c] J. Donohue, *J. Am. Chem. Soc.*, **72**, 2701 (1950); J. Donohue, "Organic Sulfur Compounds", Vol. 1, N. Kharasch, Ed., Pergamon Press, N.Y., 1961, p 1.

predict a charge redistribution that is completely consistent with that expected on the basis of the results of previous calculations for other systems.[39] The charge redistribution predicted by the EH calculations shares some but not all of the features of the ab initio redistribution. Both methods predict a weakening of the C=O bond and a strengthening of the C—N bond, but, as in the case of other hydrogen-bonded systems,[50] the EH method greatly underestimates $E_H$. The ability of the EH method to describe the effect of hydrogen bonding on the peptide linkage is clearly limited and consequently we shall not present our cyclocystine–water results.

A simple analysis of van der Waals contacts leads to the same conclusions as the EH calculations. In Table V we compare the shortest van der Waals type distances, $d$, in models I-VIII with the sums of the corresponding van der Waals radii, $d_w$. The extent to which the former are shorter than the latter can be taken as a measure of conformational energy. It follows from the degree of shortening, $d_w - d$, that models I and VII should be more stable than their counterpart models IV and VIII, respectively, and that there should be little difference in stability between models V and VI. The distances between pairs of atoms bonded to a common atom and negative $d_w - d$ values have been omitted from the table.

## Conclusions

According to the results of EH calculations on model systems, the preferred helicities of the bridging groups of 3,6-epi(dithio-, tetrathio-, and $CH_2S_2CH_2$-) 2,5-diketopiperazines are determined both by the nonbonded interactions between the bridge and the 2,5-DKP ring and by bond strengths.

In the dithio-bridged compounds, the electrostatic and covalent nonbonded interactions between the sulfur and amide carbon and nitrogen atoms favor the observed helicity (I), whereas bond strengths seem to favor the alternative helicity. The total energies indicate that the former win out and that there is a strong overall preference for the observed helicity.

In the tetrathio-bridged compounds, the interactions between the inner sulfur atoms and the amide carbon and nitrogen atoms are much weaker because the inner sulfur atoms lie at a much greater distance above the mean plane of these amide atoms than do the sulfur atoms of the dithio derivatives. The calculations suggest that the inner sulfurs of the tetrathio derivatives carry small positive charges. If this is the case, both the electrostatic and covalent nonbonded interactions between the bridging atoms and the amide carbons and nitrogens favor the observed helicity (V) slightly. Bond strengths do not clearly favor one helicity over the other. Hence, it is not surprising that the difference in total energy between the observed and alternative conformers is calculated to be negligible. However, as expected from the calculations, the outer sulfurs are skewed toward the amide carbons in the observed conformer. Therefore, by taking up $M$ helicity, the inner sulfurs permit the tetrathio bridge itself to attain a more stable staggered conformation. Nevertheless, the EH calculations suggest that the conformer in which the outer sulfurs are skewed toward the amide nitrogens and the inner sulfurs take up $P$ helicity also should be represented in nature.

In the $CH_2S_2CH_2$-bridged compounds, bond strengths are responsible for the observed helicity (VII). The electrostatic interactions between the bridging atoms and the amide carbon and nitrogen atoms favor the alternative helicity, while the combined effect of their covalent counterparts is negligible. The effect of methyl substitution at the nitrogens of cyclocystine is to increase its preference slightly for the observed helicity.

The conformational preferences inferred from the EH calculations are the same as those deduced from a classical analysis of the van der Waals interactions between the bridging atoms and the amide carbon and nitrogen atoms.

Experimental and theoretical results indicate that C=O···H—O bonding does not alter the conformational preference of any of these systems.

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# Crystal Structure and Conformation of the Cyclic Dipeptide *cyclo*-(L-Histidyl-L-aspartyl) Trihydrate[1a]

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**Abstract:** The crystal structure of *cyclo*-(L-histidyl-L-aspartyl) trihydrate has been determined by x-ray diffraction techniques, and refined to a final $R$ index of 0.056 for 1601 reflections. The molecule is in a folded conformation, with the imidazole ring facing the diketopiperazine ring. However, since the diketopiperazine ring is essentially planar, the interaction between the two rings is not as intimate as in those cyclic dipeptides in which the diketopiperazine ring is in a boat conformation with the side chain occupying an axial, or flagpole, site. Planarity of the diketopiperazine ring may be dictated by steric interactions between the imidazole ring and the aspartyl side chain. The molecule is a zwitterion, a proton having been transferred from the carboxyl group of the aspartyl side chain to the imidazole ring.

## Introduction

X-ray diffraction studies on a number of cyclic dipeptides indicate that the six-membered diketopiperazine (hereafter, DKP) ring usually adopts either a planar or a boat conformation. In the absence of steric effects due to the amino acid side chains the preferred conformation is apparently planar, as observed for *cyclo*-(L-alanyl-D-alanyl)[2] and for diketopiperazine itself, *cyclo*-(glycylglycyl).[3] Predictably, a prolyl residue, which results in a fusion between the DKP ring and the five-membered pyrrolidine ring, requires a quasi-equatorial site for the $\beta$-carbon atom and forces the DKP ring into a nonplanar conformation.[4] The favored nonplanar conformation is a boat, which permits the torsion angles about the peptide bonds C′–N to remain close to 0°.

A particularly interesting observation, first noted by Kopple and co-workers from NMR studies,[5] is that cyclic dipeptides containing an aromatic sidechain, such as phenylalanyl, tyrosyl, tryptophyl, or histidyl, tend to adopt a folded conformation with the aromatic ring facing the DKP ring. The folded conformation has been confirmed by x-ray diffraction studies on crystals of *cyclo*-(glycyl-L-tyrosyl),[6] *cyclo*-(L-seryl-L-tyrosyl),[6] and *cyclo*-(L-prolyl-D-phenylalanyl),[4a] and in the N-methylated derivatives of *cyclo*-(L-phenylalanyl-L-phenylalanyl) and *cyclo*-(L-phenylalanyl-D-phenylalanyl).[7] On the other hand, in crystals of *cyclo*-(glycyl-L-tryptophyl)[8] and *cyclo*-(L-threonyl-L-histidyl) dihydrate[9] the aromatic side chains have been found to adopt an extended conformation with no intramolecular interaction with the DKP rings.

We report here the results of a crystal-structure investigation of *cyclo*-(L-histidyl-L-aspartyl) trihydrate. Unlike the structure of *cyclo*-(L-threonyl-L-histidyl) dihydrate, the imidazole ring, which is protonated in this zwitterionic molecule, is folded back over the DKP ring.

## Experimental Section

Crystals were supplied by Professor K. D. Kopple of the Department of Chemistry, Illinois Institute of Technology. The one chosen for data collection was of irregular shape, approximately 0.3 mm in maximum dimension. X-ray data were collected using a Datex-automated General Electric diffractometer equipped with a scintillation detector and pulse-height discriminator; the radiation was Ni-filtered Cu K$\alpha$.

Crystal data are collected in Table I. Intensities for all reflections within the $\bar{h}kl$ octant of reciprocal space were measured using $\theta$–$2\theta$ scans at a scan speed of 1° (in $2\theta$) per minute, to a limiting $2\theta$ value

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