# Energetics of the Disulfide Bridge: An Ab Initio Study

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**SYNOPSIS**

The energetics of the $\chi_1^2\chi^3\chi_2^2$ portion of the disulfide bridge have been obtained from an ab initio study of diethyl disulfide. Calculations at the 3-21G* level were done on relaxed structures at every $\sim$ 30° in $\chi_1^2$ and $\chi_2^2$, and the additional energies for small $\Delta\chi^3$ were obtained. Complete $E(\chi_1^2, \chi_2^2)$ and $\chi_0^3(\chi_1^2, \chi_2^2)$ maps were computed from Fourier series expansions. These results have been used to calculate the energetics of 92 disulfide bridges in known protein structures, and to compare ab initio and molecular mechanics energies for some observed and predicted bridges. The differences found in relative energies and in $\chi_0^3$ values suggest that present energy functions give a limited description of the structural and energetic properties of the disulfide bridge. 

## INTRODUCTION

The disulfide bridge, (NH)(CO)$C^{\alpha}$H-$C^{\beta}H_2$-S-S-$C^{\beta}H_2$-$C^{\alpha}$H(NH)(CO), is one of the basic elements of the three-dimensional structures of proteins.[1] Knowledge of its conformational energies is therefore of importance in assessing the stabilities of protein structures, whether with native[2] or engineered[3-6] disulfides. While there have been several studies on the stereochemical modeling of disulfide bridges,[7-10] very few[4,8] have dealt with the energetics of such structures, and these have used rough molecular mechanics energy functions to evaluate the conformations. Recent ab initio calculations[11] have been restricted to studies of the barriers and dihedral angle of dimethyl disulfide, which provides only restricted information on the full disulfide bridge.

To reliably characterize the energetics of the main part of the disulfide bridge requires at a minimum an ab initio analysis of diethyl disulfide, which can provide the dependence of the parameters on the $C^{\beta}SSC^{\beta}$, $\chi^3$, and the two $C^{\alpha}C^{\beta}SS$, $\chi^2$, dihedral angles. As a preliminary step[12] in deriving an ab initio vibrational force field to relate SS and CS stretch frequencies of the disulfide bridge to its conformation,[13] we had calculated the energies of 20 stationary state conformers of this molecule. We have now extended these calculations to produce a $\chi_1^2$–$\chi_2^2$ energy map for $\chi_0^3$, i.e., at its minimum, and determined the dependence of this energy on small values of $\Delta\chi^3$. A map of $\chi_0^3$ as a function of $\chi_1^2$ and $\chi_2^2$ shows that this angle departs significantly from $\sim$ 90° in many regions of the conformational space. These results represent an initial step in providing an accurate basis for determining relative energies and structures of disulfide bridge conformations.

## CALCULATIONS AND RESULTS

Mapping out the entire conformation space of the disulfide bridge by ab initio calculations would be an extravagant task. This is particularly true if we were to include the dependence of energy on the $NC^{\alpha}C^{\beta}S$, $\chi^1$, dihedral angles. For the present, we assume that the latter energy is associated with the polypeptide chain, and that the dependence on $\chi_1^2$, $\chi^3$, and $\chi_2^2$ is not significantly affected by $\chi^1$. Nor do we scan the entire three-dimensional $\chi_1^2\chi^3\chi_2^2$ space. Rather, we fully optimize geometries at given $\chi_1^2\chi_2^2$, obtaining energies $E$ and $\chi_0^3$ values, and then explore $\Delta E$ as a function of small $\Delta\chi^3$ in selected regions. As we will see, there seems to be a regularity to this variation.

As in our previous studies,[12] the ab initio calculations were done with the 3-21G* basis set. In addition to the previous 20 conformers at energy minima, maxima, and saddle points, we have computed

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**Table I Energies, Dihedral Angles, and Some Geometric Parameters[a] of Conformers of Diethyl Disulfide**

| Conformer[b] | $E^c$ | $\chi_1^2$ | $\chi_0^3$ | $\chi_2^2$ | $r(C_1S)$ | $r(SS)$ | $r(SC_2)$ | $\theta(CC_1S)$ | $\theta(C_1SS)$ | $\theta(SSC_2)$ | $\theta(SC_2C)$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| TT | 0.40 | 177.6 | 86.6 | 177.6 | 1.828 | 2.044 | 1.828 | 109.0 | 102.9 | 102.9 | 109.0 |
| TD′ | 1.41 | 177.6 | 86.8 | −150.0 | 1.827 | 2.044 | 1.834 | 109.0 | 102.6 | 103.6 | 109.9 |
| TS′ | 2.09 | 176.5 | 86.9 | −123.8 | 1.828 | 2.043 | 1.838 | 109.0 | 103.0 | 104.2 | 111.5 |
| TB′ | 1.28 | 177.6 | 93.0 | −90.0 | 1.829 | 2.044 | 1.830 | 108.7 | 104.0 | 103.9 | 113.9 |
| TG′ | 0.82 | 174.8 | 97.5 | −68.6 | 1.830 | 2.046 | 1.827 | 108.7 | 103.6 | 103.7 | 114.6 |
| TA′ | 3.01 | 177.6 | 93.6 | −30.0 | 1.828 | 2.042 | 1.842 | 108.8 | 103.4 | 107.1 | 116.4 |
| TC | 4.03 | 174.0 | 90.8 | −4.7 | 1.828 | 2.039 | 1.849 | 108.9 | 103.4 | 107.8 | 116.7 |
| TA | 2.50 | 177.6 | 85.3 | 30.0 | 1.828 | 2.039 | 1.840 | 109.0 | 103.4 | 106.7 | 115.9 |
| TG | 0.20 | 177.6 | 86.6 | 69.4 | 1.828 | 2.043 | 1.828 | 109.0 | 102.7 | 103.3 | 113.6 |
| TB | 0.92 | 177.6 | 86.9 | 90.0 | 1.827 | 2.044 | 1.831 | 108.9 | 102.7 | 103.2 | 112.7 |
| TS | 1.98 | 177.1 | 87.4 | 120.0 | 1.828 | 2.043 | 1.838 | 109.0 | 103.1 | 104.1 | 111.4 |
| TD | 1.12 | 177.6 | 86.4 | 150.0 | 1.828 | 2.042 | 1.833 | 109.0 | 103.4 | 103.7 | 110.0 |
| D′D′ | 2.51 | −150.0 | 87.0 | −150.0 | 1.834 | 2.044 | 1.834 | 109.8 | 103.4 | 103.4 | 109.8 |
| D′S′ | 3.28 | −150.0 | 87.8 | −123.8 | 1.835 | 2.044 | 1.838 | 109.8 | 103.9 | 104.1 | 111.6 |
| D′B′ | 2.32 | −150.0 | 94.3 | −90.0 | 1.835 | 2.045 | 1.830 | 109.7 | 104.6 | 103.4 | 113.8 |
| D′G′ | 1.72 | −150.0 | 96.7 | −69.5 | 1.835 | 2.045 | 1.827 | 109.8 | 104.2 | 103.2 | 114.5 |
| D′A′ | 4.06 | −150.0 | 92.9 | −30.0 | 1.835 | 2.042 | 1.843 | 109.9 | 104.0 | 106.7 | 116.4 |
| D′C | 5.14 | −150.0 | 89.8 | −4.7 | 1.835 | 2.039 | 1.850 | 109.9 | 104.2 | 107.5 | 116.7 |
| D′A | 3.66 | −150.0 | 86.0 | 30.0 | 1.835 | 2.039 | 1.840 | 109.9 | 104.1 | 106.5 | 116.0 |
| D′G | 1.23 | −150.0 | 86.7 | 68.2 | 1.834 | 2.045 | 1.827 | 110.0 | 103.4 | 103.0 | 113.6 |
| D′B | 2.02 | −150.0 | 87.3 | 90.0 | 1.834 | 2.045 | 1.831 | 109.8 | 103.4 | 102.9 | 112.7 |
| D′S | 3.13 | −150.0 | 88.3 | 117.4 | 1.835 | 2.044 | 1.838 | 109.8 | 103.9 | 103.9 | 111.5 |
| D′D | 2.20 | −150.0 | 87.5 | 150.0 | 1.835 | 2.043 | 1.833 | 109.8 | 104.1 | 103.4 | 109.6 |
| S′S′ | 3.98 | −125.5 | 88.6 | −125.5 | 1.839 | 2.043 | 1.839 | 111.4 | 104.4 | 104.4 | 111.4 |
| S′B′ | 2.79 | −125.5 | 92.3 | −90.0 | 1.839 | 2.044 | 1.829 | 111.5 | 105.0 | 103.7 | 113.7 |
| S′G′ | 2.42 | −125.5 | 95.6 | −74.4 | 1.839 | 2.045 | 1.826 | 111.8 | 105.1 | 104.0 | 114.4 |
| S′A′ | 4.82 | −125.5 | 95.0 | −30.0 | 1.839 | 2.043 | 1.842 | 112.1 | 104.7 | 107.4 | 116.4 |
| S′C | 6.09 | −115.0 | 97.3 | −6.7 | 1.838 | 2.041 | 1.849 | 113.2 | 104.7 | 108.6 | 117.0 |
| S′A | 4.27 | −125.5 | 88.2 | 30.0 | 1.839 | 2.039 | 1.840 | 111.7 | 104.7 | 106.9 | 115.9 |
| S′G | 1.93 | −123.9 | 86.5 | 68.2 | 1.838 | 2.044 | 1.827 | 111.5 | 104.1 | 103.4 | 113.6 |
| S′B | 2.78 | −125.5 | 87.8 | 90.0 | 1.838 | 2.044 | 1.831 | 111.5 | 104.1 | 103.4 | 112.7 |
| S′S | 3.83 | −125.5 | 89.2 | 118.6 | 1.839 | 2.043 | 1.838 | 111.5 | 104.5 | 104.3 | 111.4 |
| S′D | 2.84 | −125.5 | 88.0 | 150.0 | 1.839 | 2.042 | 1.833 | 111.4 | 104.6 | 103.6 | 109.6 |
| B′B′ | 3.00 | −90.0 | 106.5 | −90.0 | 1.831 | 2.051 | 1.831 | 114.9 | 104.9 | 104.9 | 114.9 |
| B′G′ | 2.73 | −90.0 | 111.5 | −69.5 | 1.831 | 2.053 | 1.828 | 115.0 | 103.9 | 104.3 | 115.4 |
| B′A′ | 4.88 | −90.0 | 107.6 | −30.0 | 1.831 | 2.048 | 1.843 | 115.0 | 103.7 | 107.8 | 117.2 |
| B′C | 5.41 | −90.0 | 102.5 | −4.7 | 1.831 | 2.043 | 1.849 | 114.6 | 103.7 | 108.5 | 117.1 |
| B′A | 3.16 | −90.0 | 91.6 | 30.0 | 1.829 | 2.041 | 1.840 | 113.8 | 103.9 | 107.4 | 115.8 |
| B′G | 1.12 | −90.0 | 93.5 | 68.2 | 1.830 | 2.045 | 1.828 | 113.9 | 103.7 | 104.3 | 113.4 |
| B′B | 1.86 | −90.0 | 94.6 | 90.0 | 1.830 | 2.045 | 1.832 | 113.9 | 103.5 | 104.1 | 112.6 |
| B′S | 2.64 | −90.0 | 92.4 | 117.4 | 1.829 | 2.043 | 1.839 | 113.6 | 103.7 | 104.8 | 111.5 |
| B′D | 1.75 | −90.0 | 90.6 | 150.0 | 1.829 | 2.042 | 1.833 | 113.6 | 104.1 | 104.4 | 109.5 |
| G′G′ | 2.36 | −71.0 | 114.5 | −71.0 | 1.829 | 2.056 | 1.829 | 115.5 | 103.7 | 103.7 | 115.5 |
| G′A′ | 4.46 | −69.5 | 109.8 | −30.0 | 1.829 | 2.047 | 1.844 | 115.5 | 103.3 | 107.3 | 117.3 |
| G′C | 4.97 | −70.5 | 105.7 | −12.6 | 1.829 | 2.045 | 1.849 | 115.2 | 103.4 | 108.2 | 117.3 |
| G′A | 2.70 | −69.5 | 94.4 | 30.0 | 1.827 | 2.042 | 1.841 | 114.3 | 103.8 | 107.3 | 115.8 |
| G′G | 0.66 | −69.1 | 98.0 | 68.6 | 1.827 | 2.047 | 1.829 | 114.6 | 103.5 | 103.8 | 113.4 |
| G′B | 1.30 | −69.5 | 97.6 | 90.0 | 1.827 | 2.046 | 1.833 | 114.6 | 103.3 | 103.7 | 112.5 |
| G′S | 2.06 | −69.5 | 94.6 | 117.4 | 1.827 | 2.044 | 1.839 | 114.3 | 103.6 | 104.6 | 111.4 |
| G′D | 1.30 | −69.5 | 95.0 | 150.0 | 1.827 | 2.044 | 1.834 | 114.5 | 104.0 | 104.2 | 109.4 |
| A′A′ | 6.64 | −30.0 | 105.0 | −30.0 | 1.845 | 2.042 | 1.845 | 117.3 | 107.1 | 107.1 | 117.3 |
| A′C | 6.92 | −30.0 | 98.6 | −4.7 | 1.844 | 2.038 | 1.850 | 116.7 | 107.0 | 107.9 | 117.1 |
| A′A | 4.83 | −30.0 | 90.2 | 30.0 | 1.842 | 2.038 | 1.839 | 115.9 | 107.1 | 107.1 | 115.9 |
| A′G | 2.90 | −30.0 | 93.8 | 68.2 | 1.843 | 2.043 | 1.828 | 116.5 | 106.9 | 103.7 | 113.4 |

**Table I** (*Continued*)

| Conformer[b] | $E$[c] | $\chi_1^2$ | $\chi_0^3$ | $\chi_2^2$ | $r(C_1S)$ | $r(SS)$ | $r(SC_2)$ | $\theta(CC_1S)$ | $\theta(C_1SS)$ | $\theta(SSC_2)$ | $\theta(SC_2C)$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A′B | 3.62 | −30.0 | 93.8 | 90.0 | 1.843 | 2.042 | 1.832 | 116.5 | 106.8 | 103.5 | 112.5 |
| A′S | 4.32 | −30.0 | 91.8 | 117.4 | 1.842 | 2.041 | 1.837 | 116.1 | 106.9 | 104.3 | 111.3 |
| A′D | 3.42 | −30.0 | 92.0 | 150.0 | 1.842 | 2.041 | 1.832 | 116.1 | 107.2 | 103.9 | 109.4 |
| CC | 7.54 | −11.6 | 95.7 | −11.6 | 1.849 | 2.035 | 1.849 | 116.9 | 107.7 | 107.7 | 116.9 |
| CA | 5.94 | −4.7 | 88.5 | 30.0 | 1.848 | 2.036 | 1.838 | 116.4 | 108.0 | 107.1 | 115.9 |
| CG | 3.93 | −5.2 | 90.8 | 68.3 | 1.850 | 2.039 | 1.828 | 116.8 | 107.7 | 103.8 | 113.5 |
| CB | 4.70 | −4.7 | 90.5 | 90.0 | 1.850 | 2.039 | 1.832 | 116.7 | 107.6 | 103.7 | 112.6 |
| CS | 5.52 | −1.8 | 89.3 | 116.5 | 1.849 | 2.038 | 1.837 | 116.5 | 107.7 | 104.5 | 111.5 |
| CD | 4.56 | −4.7 | 90.2 | 150.0 | 1.849 | 2.038 | 1.832 | 116.5 | 108.0 | 104.0 | 109.5 |
| AA | 4.42 | 30.0 | 84.6 | 30.0 | 1.839 | 2.036 | 1.839 | 115.8 | 107.0 | 107.0 | 115.8 |
| AG | 2.36 | 30.0 | 85.1 | 68.2 | 1.828 | 2.040 | 1.840 | 113.6 | 103.7 | 106.6 | 115.9 |
| AB | 3.19 | 30.0 | 86.1 | 90.0 | 1.840 | 2.040 | 1.832 | 116.0 | 106.6 | 103.7 | 112.7 |
| AS | 4.10 | 30.0 | 87.0 | 117.4 | 1.840 | 2.039 | 1.838 | 115.9 | 106.8 | 104.5 | 111.5 |
| AD | 3.10 | 30.0 | 85.9 | 150.0 | 1.840 | 2.039 | 1.832 | 115.8 | 106.9 | 104.0 | 109.6 |
| GG | 0.00 | 69.4 | 86.3 | 69.4 | 1.827 | 2.045 | 1.827 | 113.5 | 103.1 | 103.1 | 113.5 |
| GB | 0.74 | 68.2 | 86.9 | 90.0 | 1.827 | 2.045 | 1.831 | 113.6 | 103.0 | 103.0 | 112.7 |
| GS | 1.80 | 68.2 | 87.2 | 119.9 | 1.827 | 2.043 | 1.838 | 113.6 | 103.5 | 104.0 | 111.4 |
| GD | 0.93 | 68.2 | 86.5 | 150.0 | 1.828 | 2.043 | 1.832 | 113.6 | 103.7 | 103.5 | 109.6 |
| BB | 1.53 | 90.0 | 87.4 | 90.0 | 1.831 | 2.045 | 1.831 | 112.7 | 103.0 | 103.0 | 112.7 |
| BS | 2.64 | 90.0 | 88.2 | 117.4 | 1.831 | 2.044 | 1.838 | 112.8 | 103.4 | 104.0 | 111.5 |
| BD | 1.86 | 90.0 | 87.4 | 150.0 | 1.831 | 2.044 | 1.833 | 112.7 | 103.6 | 103.4 | 109.7 |
| SS | 3.69 | 118.7 | 89.8 | 118.7 | 1.838 | 2.042 | 1.838 | 111.4 | 104.4 | 104.4 | 111.4 |
| SD | 2.71 | 117.4 | 88.4 | 150.0 | 1.837 | 2.042 | 1.832 | 111.6 | 104.5 | 103.8 | 109.6 |
| DD | 1.79 | 150.0 | 87.1 | 150.0 | 1.833 | 2.021 | 1.833 | 109.7 | 104.0 | 104.0 | 109.7 |

[a] Bond lengths, $r$, in Å. Bond angles, $\theta$, in degrees. Dihedral angles, $\chi_1^2 = CC_1SS$, $\chi_0^3 = C_1SSC_2$, $\chi_2^2 = SSC_2C$, in degrees.

[b] Conformer, designated by $\chi_1^2\chi_2^2$. $T = 180°$, $D = 150°$, $S = 120°$, $B = 90°$, $G = 60°$, $A = 30°$, $C = 0°$ (for $\chi^3 > 0$). Prime indicates negative angle.

[c] Energy, in kcal/mole.

fully relaxed structures at the remaining 30° intervals in $\chi_1^2$ and $\chi_2^2$, for a total of 78 structures. (Calculations were for $\chi^3 > 0$; these results apply to $\chi^3 < 0$ if the signs of $\chi_1^2$, $\chi^3$, and $\chi_2^2$ are reversed.) The resulting values of $E$ (with respect to the global minimum), $\chi_0^3$, and selected geometric parameters are given in Table I. The conformations are designated by the closest values of the $\chi^2$ dihedral angle: 0°(C), 30°(A), 60°(G), 90°(B), 120°(S), 150°(D), 180°(T), and their negative values A′, G′, B′, S′, and D′.

In order to present a relatively accurate energy contour map as a function of $\chi_1^2$ and $\chi_2^2$, we make use of the fact that $E$ is a periodic function of these variables and can therefore be represented as a two-dimensional Fourier series whose coefficients can be determined from the calculated energies (see Appendix). The energy contours are then found by a program that uses the Fourier series as an analytic function to search by 0.5°–1.0° steps along the tangents and with 0.001 kcal accuracy along the normals of the contours. The resulting map is given in Figure 1, with energies relative to the GG minimum, $E_{GG}$; the exact energy at any $\chi_1^2\chi_2^2$ for $\chi_0^3$ can be obtained from Eq. (A4) using the coefficients of Table AI. The same procedure can be used for $\chi_0^3$, the contours being determined by a search with 0.01° accuracy along the normals. The resulting map is given in Figure 2, with exact values obtainable from Eq. (A4) using the coefficients of Table AII.

In order to determine the additional energy associated with a departure from $\chi_0^3$, we have calculated ab initio energies at selected $\chi_1^2\chi_2^2$ values for $\Delta\chi^3 = \pm 10°$ and $\pm 20°$. The underlying assumption is that the $\chi_1^2\chi_2^2$ dependence for such small $\Delta\chi^3$ (which is in the range observed for 92 protein disulfide bridges[14]) is not significant. Calculations were done for 15 conformers that span a range of $E$ and $\chi_0^3$. We found that $E(\Delta\chi^3)$ depended essentially only on $\chi_0^3$: for 7 conformers with $\chi_0^3 < 90°$ (GA, GG, TD, TT, TS′, BD, and SD), $E(\Delta\chi^3)$ had a value at any $\Delta\chi^3$ that was independent of $E_{GG}$ (which varied from 0.00 to 2.71 kcal/mole); the same was true for 8 conformers with $\chi_0^3 > 90°$ (S′B′, B′G, G′S,

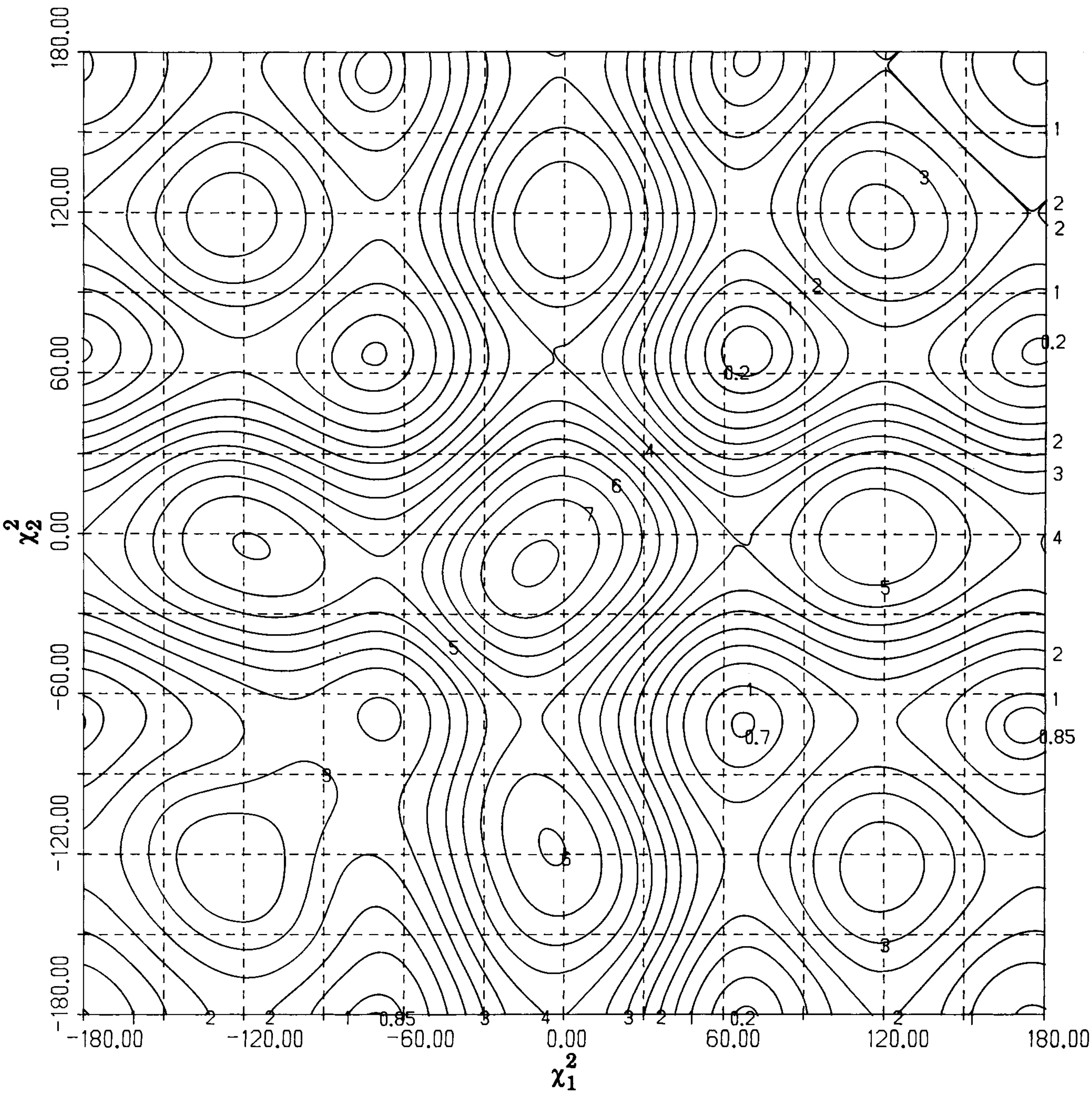


**Figure 1.** Energies (in kcal/mole) of relaxed diethyl disulfide structures as a function of $\chi_1^2$ and $\chi_2^2$. Contours are at intervals of 0.5 kcal/mole, except where otherwise designated.

S′A′, S′G′, G′B, G′G, and G′G′), although the specific $E(\Delta\chi^3)$ is different ($E_{GG}$ varied from 0.66 to 4.82 kcal/mole for these conformers). By averaging the energies of the conformers in each group of $\Delta\chi^3 = \pm 10°$ and $\pm 20°$, and fitting the energy variation with a three-term Fourier series, we can accurately obtain $E(\Delta\chi^3)$ for intermediate $\Delta\chi^3$. These values are given in Table II, together with average deviations at 5° intervals. As can be seen, the latter are $\leq 0.2$ kcal/mole for all $\Delta\chi^3$ except $\Delta\chi^3 = -20°$, $-25°$, and $-30°$ for $\chi_0^3 > 90°$, suggesting that the assumption that $E(\Delta\chi^3)$ has a small dependence on $\chi_1^2\chi_2^2$ is substantially valid for most $\chi_0^3$, particularly when $|\Delta\chi^3| < 20°$.

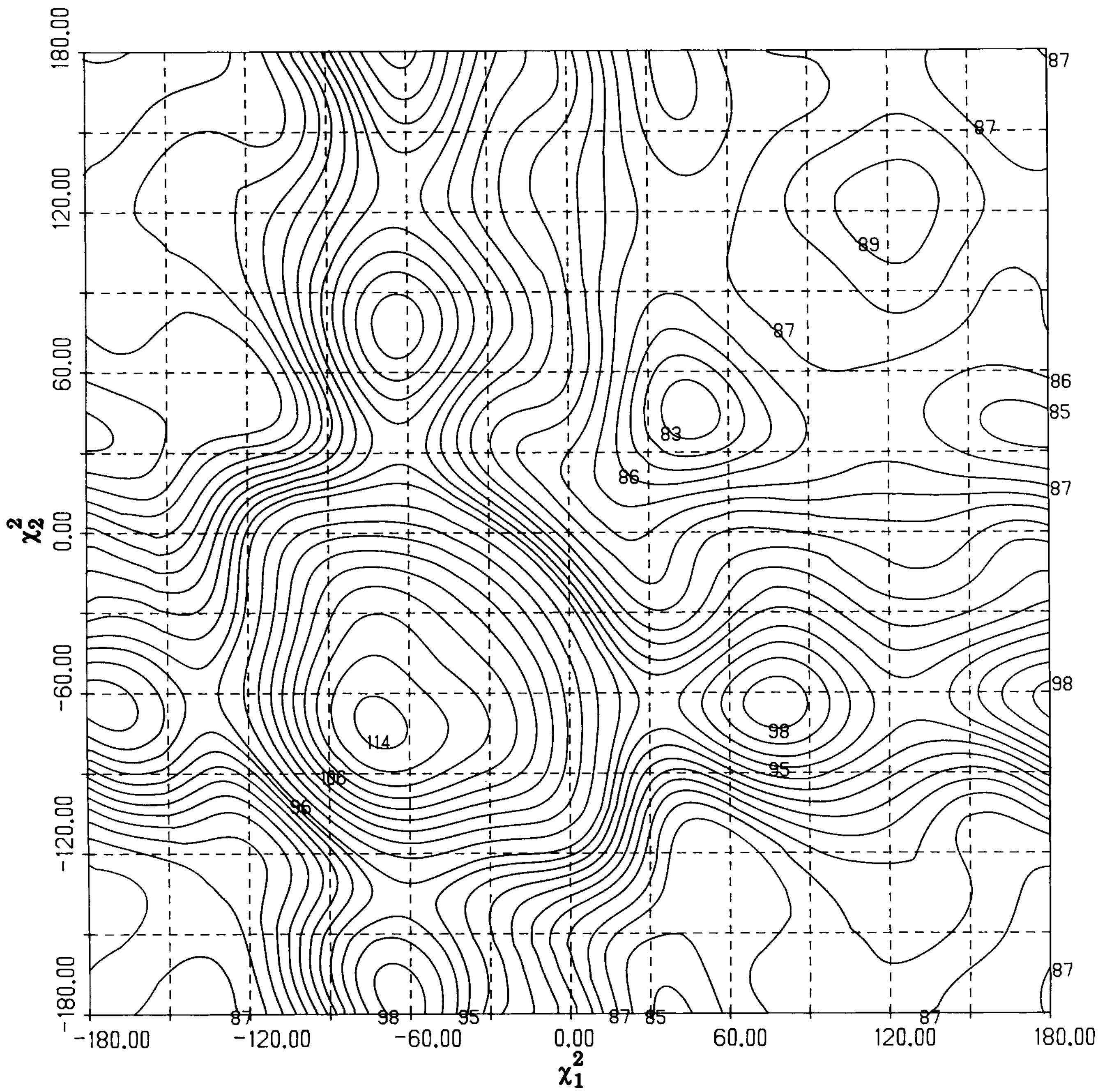


**Figure 2.** $\chi_0^3$ (in degrees) of relaxed diethyl disulfide structures as a function of $\chi_1^2$ and $\chi_2^2$. Contours are at intervals of 1°, except above 96°, where the interval is 2°.

## DISCUSSION

The results represented by Figures 1 and 2 provide a view of the energetic and conformational landscape of the $\chi_1^2\chi^3\chi_2^2$ portion of the disulfide bridge. The maps are of course symmetrical about the $\chi_1^2$–$\chi_2^2$ diagonal. It can be seen from Figure 1 that the 20 stationary structures are divided into three groups: 6 conformers are at energy minima (GG, GT, TT, GG′, TG′, and G′G′), another 6 conformers, SS, SS′, S′S′, CS, CS′, and CC, are at maxima, and the remainder are all at transition states, surrounded by two minima and two maxima. This clearly shows that the conformer G′S′, which could not be fully optimized in the region around −74° and −125°,[12] is not at a saddle point, and a transition structure

**Table II Energy Variation[a] with $\Delta\chi^3$ [b] from $\chi^3_0$ for Diethyl Disulfide**

| $\chi^3_0 < 90°$ | | | | $\chi^3_0 > 90°$ | | | |
|---|---|---|---|---|---|---|---|
| $\Delta\chi^3$ | $E^c$ | $\Delta\chi^3$ | $E^d$ | $\Delta\chi^3$ | $E^e$ | $\Delta\chi^3$ | $E^f$ |
| 1 | 0.00 | −1 | 0.00 | 1 | 0.01 | −1 | 0.01 |
| 2 | 0.01 | −2 | 0.01 | 2 | 0.02 | −2 | 0.02 |
| 3 | 0.02 | −3 | 0.02 | 3 | 0.04 | −3 | 0.04 |
| 4 | 0.04 | −4 | 0.04 | 4 | 0.06 | −4 | 0.07 |
| 5 | 0.07 | −5 | 0.07 | 5 | 0.10 | −5 | 0.11 |
| 6 | 0.10 | −6 | 0.11 | 6 | 0.14 | −6 | 0.15 |
| 7 | 0.13 | −7 | 0.15 | 7 | 0.18 | −7 | 0.21 |
| 8 | 0.18 | −8 | 0.20 | 8 | 0.24 | −8 | 0.28 |
| 9 | 0.22 | −9 | 0.25 | 9 | 0.30 | −9 | 0.35 |
| 10 | 0.27 | −10 | 0.31 | 10 | 0.36 | −10 | 0.44 |
| 11 | 0.33 | −11 | 0.38 | 11 | 0.43 | −11 | 0.53 |
| 12 | 0.38 | −12 | 0.45 | 12 | 0.51 | −12 | 0.63 |
| 13 | 0.45 | −13 | 0.53 | 13 | 0.59 | −13 | 0.75 |
| 14 | 0.51 | −14 | 0.62 | 14 | 0.67 | −14 | 0.87 |
| 15 | 0.58 | −15 | 0.72 | 15 | 0.76 | −15 | 1.01 |
| 16 | 0.66 | −16 | 0.82 | 16 | 0.85 | −16 | 1.15 |
| 17 | 0.74 | −17 | 0.93 | 17 | 0.95 | −17 | 1.30 |
| 18 | 0.82 | −18 | 1.04 | 18 | 1.05 | −18 | 1.47 |
| 19 | 0.90 | −19 | 1.17 | 19 | 1.15 | −19 | 1.64 |
| 20 | 0.99 | −20 | 1.29 | 20 | 1.26 | −20 | 1.82 |
| 21 | 1.07 | −21 | 1.43 | 21 | 1.37 | −21 | 2.02 |
| 22 | 1.16 | −22 | 1.57 | 22 | 1.48 | −22 | 2.22 |
| 23 | 1.26 | −23 | 1.72 | 23 | 1.59 | −23 | 2.43 |
| 24 | 1.35 | −24 | 1.87 | 24 | 1.70 | −24 | 2.65 |
| 25 | 1.45 | −25 | 2.04 | 25 | 1.82 | −25 | 2.89 |
| 26 | 1.55 | −26 | 2.20 | 26 | 1.93 | −26 | 3.13 |
| 27 | 1.65 | −27 | 2.38 | 27 | 2.05 | −27 | 3.38 |
| 28 | 1.75 | −28 | 2.55 | 28 | 2.17 | −28 | 3.63 |
| 29 | 1.85 | −29 | 2.74 | 29 | 2.29 | −29 | 3.90 |
| 30 | 1.95 | −30 | 2.93 | 30 | 2.41 | −30 | 4.17 |

[a] In kcal/mole.
[b] In degrees.
[c] With average deviations: 5°: ±0.00; 10°: ±0.01; 15°: ±0.02; 20°: ±0.02; 25°: ± 0.03; 30°: ±0.06.
[d] With average deviations: −5°: ±0.01; −10°: ±0.01; −15°: ±0.04; −20°: ±0.08; −25°: ±0.13; −30°: ±0.20.
[e] With average deviations: 5°: ±0.02; 10°: ±0.04; 15°: ±0.06; 20°: ±0.10; 25°: ±0.13; 30°: ±0.15.
[f] With average deviations: −5°: ±0.01, −10°: ±0.05; −15°: ±0.12; −20°: ±0.23; −25°: ±0.38; −30°: ±0.58.

predicted by the Fourier series may be located in the region around −72°, −99°. Calculations on a finer grid are not likely to be very helpful, both because no uncertain region was found in the map and the higher term Fourier coefficients are already very small. The maps also show an interesting relationship between $E(\chi^2_1, \chi^2_2)$ and $\chi^3_0$. We see from Figure 2 that $\chi^3_0$ is quite variable, taking values from < 83° to > 114°. It might be thought that the departure from the expected equilibrium value near 90° (86.3° for the GG conformer, Table I; 87.4° for dimethyl disulfide[11]) could be attributed to nonbonded interactions in the bridge, which would imply that highly "distorted" $\chi^3$ should be associated with high energies. This is clearly not the case. The largest $\chi^3_0$, > 114° at $\chi^2_1$, $\chi^2_2$ ~ −70°, −70°, is associated with an energy of ~2.5 kcal/mole, whereas energies of > 7.5 kcal/mole (at ~ −10°, −10°) have $\chi^3_0$ ~ 96°. Similarly, energies of ~ 0.7 kcal/mole can be associated with $\chi^3_0$ ~ 97.5° (at ~ 69°, −70°) as well as $\chi^3_0$ in the range of ~ 83.5°–87.3° (near ~ 70°, 70°). It would seem that these results reflect the possibility that interactions occur between the $\chi^2_1$, $\chi^3$, and $\chi^2_2$ torsions, a feature not taken into account by calculations based on simple torsion energy functions.[4,8]

As noted above, the energy associated with small deviations from $\chi_0^3$, $E(\Delta\chi^3)$, depend essentially only on $\chi_0^3$, indicating that the $E(\chi_1^2, \chi_2^2)$ surface is not changed significantly for small $\Delta\chi^3$. To assess this as well as other aspects of the energetics of disulfide bridges in proteins, we have computed the various energies for the 92 S-S bridges that we studied previously.[14] The results are given in Table III, and the positions of these structures on a $E(\chi_1^2, \chi_2^2)$ map are shown in Figure 3. The following will be seen: (1) Many of the structures fall in the low energy regions of the $E(\chi_1^2, \chi_2^2)$ map: 37% have energies of < 1 kcal/mole and 77% have energies of < 2 kcal/mole. All of them fall in the 2 kcal contours centered at 5 minimum energy structures, and the numbers in each region seem fairly consistent with their relative energies: GG(29), GT(16), TT(7), GG′(13), and TG′(7), although such energy relationships may be slightly different at higher levels of theory. (2) $E(\Delta\chi^3)$ is generally fairly small: for 65% of the structures, this energy is < 0.2 kcal/mole and for 86% of the structures it is < 0.5 kcal/mole. (3) There is no apparent correlation between $E(\Delta\chi^3)$ and $\chi_0^3$, either for small $\chi_0^3$ [cf. $\chi_0^3$ = 82.9° and $E(\Delta\chi^3)$ = 0.00 for no. 43 with $\chi_0^3$ = −82.7° and $E(\Delta\chi^3)$ = 0.97 for no. 44) or large $\chi_0^3$ [cf. $\chi_0^3$ = 97.1°

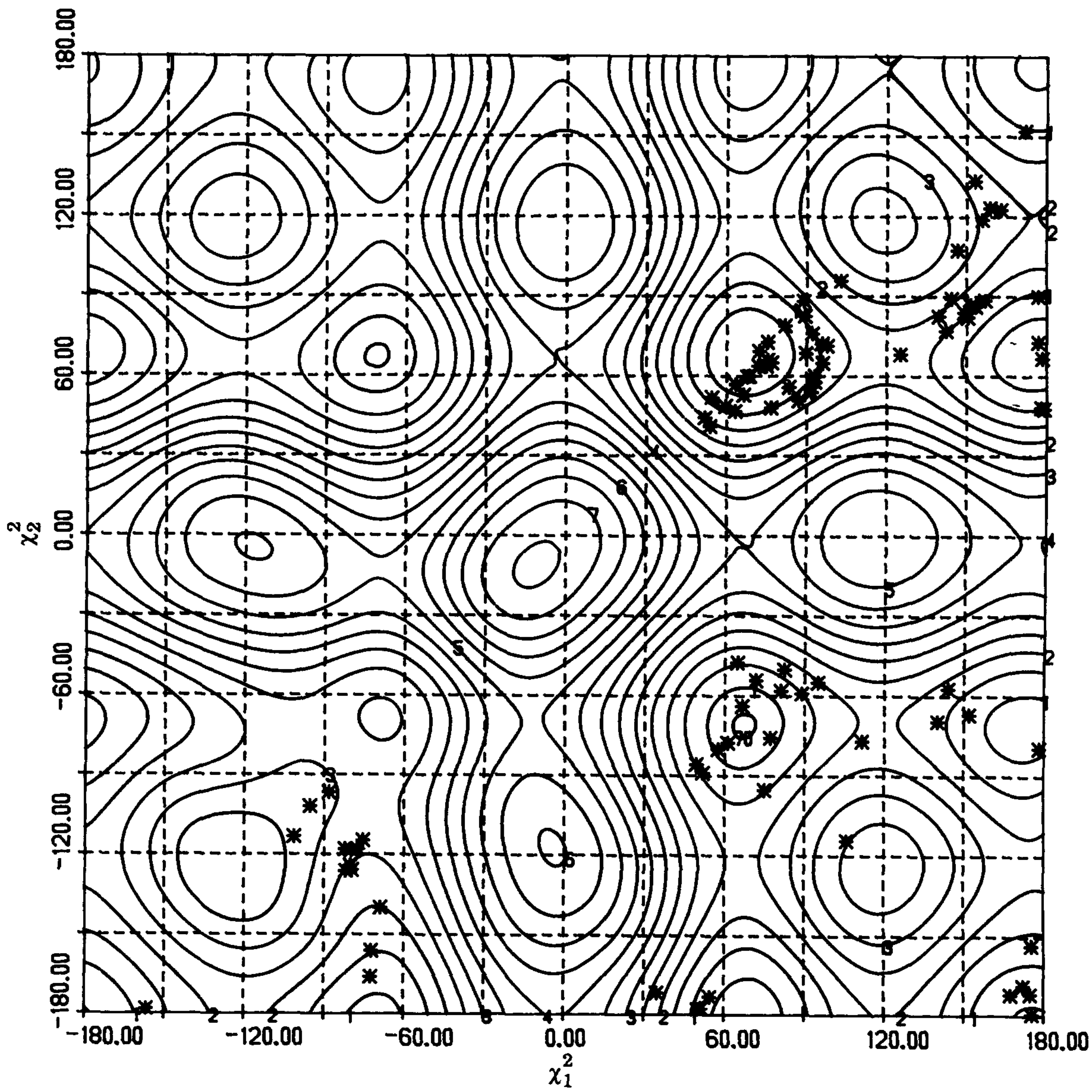


**Figure 3.** Locations of 92 disulfide bridges of known protein structures on $E(\chi_1^2, \chi_2^2)$ map.

**Table III Energies of 92 Disulfide Bridges in Proteins**

| No. | Code[a] | Cys–Cys | $\chi_1^{2b}$ | $\chi_2^{2b}$ | $E(\chi_1^2, \chi_2^2)^c$ | $\chi^{3b}$ | $\chi_0^{3b}$ | $E(\chi^3)^c$ | $E(\chi^2, \chi^3)$ |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 1ACX | 34–43 | −101.4 | −113.3 | 3.35 | 108.0 | 92.1 | 0.85 | 4.20 |
| 2 | 1ACX | 83–88 | −50.2 | 177.9 | 0.87 | −76.8 | −85.5 | 0.23 | 1.10 |
| 3 | 2AZA | A3–A26 | −60.0 | −68.6 | 0.17 | −96.9 | −85.6 | 0.35 | 0.52 |
| 4 | 2AZA | B3–B26 | −69.3 | −72.3 | 0.07 | −82.4 | −86.6 | 0.05 | 0.12 |
| 5 | 1REI | A23–A88 | −153.1 | −133.3 | 2.26 | −91.0 | −88.0 | 0.02 | 2.28 |
| 6 | 1REI | B23–B88 | 123.3 | 158.7 | 2.28 | 81.1 | 87.9 | 0.14 | 2.42 |
| 7 | 2RHE | 22–89 | 122.6 | 162.8 | 2.16 | 87.3 | 87.7 | 0.00 | 2.16 |
| 8 | 5CPA | 138–161 | 144.0 | −57.2 | 1.81 | 92.9 | 95.2 | 0.02 | 1.83 |
| 9 | 4CHA | A42–A58 | −150.4 | −82.1 | 1.41 | −87.6 | −87.1 | 0.00 | 1.41 |
| 10 | 4CHA | A136–A201 | −114.7 | −75.5 | 2.64 | 108.9 | 98.8 | 0.37 | 3.01 |
| 11 | 4CHA | A168–A182 | 153.3 | −175.5 | 1.30 | −75.4 | −86.7 | 0.40 | 1.70 |
| 12 | 4CHA | A191–A220 | 54.7 | −173.1 | 0.70 | 76.3 | 86.0 | 0.29 | 0.99 |
| 13 | 4CHA | B42–B58 | −149.1 | −84.0 | 1.58 | −88.9 | −87.2 | 0.00 | 1.58 |
| 14 | 4CHA | B136–B201 | −118.0 | −77.5 | 2.58 | 101.3 | 97.1 | 0.07 | 2.65 |
| 15 | 4CHA | B168–B182 | 156.7 | 178.3 | 1.20 | −78.1 | −86.9 | 0.24 | 1.44 |
| 16 | 4CHA | B191–B220 | 51.3 | −177.5 | 0.80 | 72.8 | 85.6 | 0.51 | 1.31 |
| 17 | 2CGA | A42–A58 | −144.3 | −89.2 | 2.09 | −92.2 | −87.6 | 0.06 | 2.15 |
| 18 | 2CGA | A136–A201 | −124.2 | −80.2 | 2.47 | 99.1 | 94.9 | 0.07 | 2.54 |
| 19 | 2CGA | A168–A182 | 168.5 | −172.4 | 0.70 | −84.7 | −86.9 | 0.01 | 0.71 |
| 20 | 2CGA | A191–B220 | 178.2 | −79.4 | 0.92 | 100.1 | 96.1 | 0.07 | 0.99 |
| 21 | 2CGA | B42–B58 | −142.2 | −77.0 | 1.54 | −98.6 | −87.2 | 0.35 | 1.89 |
| 22 | 2CGA | B136–B201 | −126.2 | −81.9 | 2.48 | 105.4 | 94.2 | 0.44 | 2.92 |
| 23 | 2CGA | B168–B182 | 171.7 | −175.0 | 0.60 | −74.2 | −87.0 | 0.52 | 1.12 |
| 24 | 2CGA | B191–B220 | −176.9 | −72.7 | 0.19 | −89.3 | −86.9 | 0.01 | 0.20 |
| 25 | 1CRN | 3–40 | −72.7 | −75.4 | 0.17 | −80.4 | −86.9 | 0.13 | 0.30 |
| 26 | 1CRN | 4–32 | −82.8 | −118.0 | 2.67 | 105.6 | 95.9 | 0.34 | 3.01 |
| 27 | 1CRN | 16–26 | −93.1 | −58.0 | 1.11 | −86.4 | −86.9 | 0.00 | 1.11 |
| 28 | 3EBX | 3–24 | −67.8 | −59.8 | 0.17 | −85.4 | −85.5 | 0.00 | 0.17 |
| 29 | 3EBX | 17–41 | −54.1 | −91.8 | 1.25 | −82.7 | −86.7 | 0.04 | 1.29 |
| 30 | 3EBX | 43–54 | 66.7 | 178.5 | 0.16 | 83.6 | 86.7 | 0.02 | 0.18 |
| 31 | 3EBX | 55–60 | 88.9 | 88.1 | 1.36 | 85.6 | 87.1 | 0.00 | 1.36 |
| 32 | 3GRS | 58–63 | 79.9 | 118.4 | 2.60 | −133.4 | −96.5 | 3.22 | 5.82 |
| 33 | 3FAB | L22–L87 | 118.9 | 155.8 | 2.43 | 110.5 | 88.0 | 1.21 | 3.64 |
| 34 | 3FAB | L136–L195 | 179.4 | −175.6 | 0.45 | −94.4 | −87.0 | 0.15 | 0.60 |
| 35 | 3FAB | H22–H95 | 171.9 | −167.6 | 0.66 | −66.1 | −86.7 | 1.37 | 2.03 |
| 36 | 3FAB | H144–H200 | −171.9 | −152.0 | 1.05 | −79.7 | −86.3 | 0.13 | 1.18 |
| 37 | 3FAB | L213–H220 | 111.6 | −76.9 | 2.10 | 166.8 | 94.7 | 5.83 | 7.93 |
| 38 | 3INS | A6–A11 | −72.5 | −166.0 | 1.21 | 104.3 | 98.2 | 0.14 | 1.35 |
| 39 | 3INS | C6–C11 | −72.3 | −156.1 | 1.53 | 108.4 | 97.4 | 0.43 | 1.96 |
| 40 | 3INS | A7–B7 | 51.9 | −88.9 | 1.44 | 98.2 | 91.8 | 0.16 | 1.60 |
| 41 | 3INS | A20–B19 | −48.7 | −59.6 | 0.87 | −78.8 | −83.5 | 0.06 | 0.93 |
| 42 | 3INS | C7–D7 | 57.4 | −80.0 | 0.94 | 98.7 | 95.0 | 0.05 | 0.99 |
| 43 | 3INS | C20–D19 | −41.1 | −53.8 | 1.66 | −83.0 | −82.9 | 0.00 | 1.66 |
| 44 | 1LZ1 | 6–128 | −51.9 | −44.3 | 1.50 | −65.3 | −82.7 | 0.97 | 2.47 |
| 45 | 1LZ1 | 30–116 | −95.7 | −72.3 | 1.08 | −95.7 | −87.2 | 0.20 | 1.28 |
| 46 | 1LZ1 | 65–81 | 81.1 | −58.1 | 1.23 | 95.0 | 98.3 | 0.05 | 1.28 |
| 47 | 1LZ1 | 77–95 | 178.0 | 48.3 | 0.95 | 83.0 | 85.2 | 0.01 | 0.96 |
| 48 | 2ALP | 42–58 | −156.9 | −88.5 | 1.44 | −88.4 | −87.0 | 0.00 | 1.44 |
| 49 | 2ALP | 137–159 | −96.6 | −88.2 | 3.05 | 102.5 | 103.9 | 0.01 | 3.06 |
| 50 | 1SN3 | 12–65 | −63.8 | 66.5 | 0.75 | 88.5 | 98.1 | 0.40 | 1.15 |
| 51 | 1SN3 | 16–41 | 97.7 | 71.5 | 1.16 | 91.4 | 87.2 | 0.05 | 1.21 |
| 52 | 1SN3 | 25–46 | −54.5 | −51.9 | 0.86 | −76.3 | −83.2 | 0.14 | 1.00 |
| 53 | 1SN3 | 29–48 | 63.5 | 56.9 | 0.32 | 74.6 | 84.7 | 0.31 | 0.63 |
| 54 | 1NXB | 3–24 | −96.1 | −65.0 | 1.07 | −82.4 | −87.1 | 0.06 | 1.13 |

**Table III** (*Continued*)

| No. | Code[a] | Cys–Cys | $\chi_1^{2b}$ | $\chi_2^{2b}$ | $E(\chi_1^2, \chi_2^2)^c$ | $\chi^{3b}$ | $\chi_0^{3b}$ | $E(\chi^3)^c$ | $E(\chi^2, \chi^3)$ |
|---|---|---|---|---|---|---|---|---|---|
| 55 | 1NXB | 17–41 | −63.8 | −74.9 | 0.16 | −84.3 | −86.4 | 0.01 | 0.17 |
| 56 | 1NXB | 43–54 | 90.1 | 176.9 | 0.92 | 84.5 | 87.0 | 0.01 | 0.93 |
| 57 | 1NXB | 55–60 | 82.5 | 89.3 | 1.10 | 86.6 | 87.1 | 0.00 | 1.10 |
| 58 | 2OVO | 8–38 | −46.8 | −63.2 | 0.93 | −75.6 | −83.9 | 0.21 | 1.14 |
| 59 | 2OVO | 16–35 | −55.8 | −83.5 | 0.74 | −79.8 | −86.5 | 0.13 | 0.87 |
| 60 | 2OVO | 24–56 | 64.7 | −47.5 | 1.57 | 98.9 | 96.2 | 0.03 | 1.60 |
| 61 | 9PAP | 22–63 | 85.8 | −49.4 | 1.47 | −88.5 | −92.4 | 0.06 | 1.53 |
| 62 | 9PAP | 56–95 | −95.3 | 75.0 | 1.46 | 97.4 | 92.7 | 0.09 | 1.55 |
| 63 | 9PAP | 153–200 | 171.6 | −34.9 | 2.31 | −85.2 | −85.0 | 0.00 | 2.31 |
| 64 | 1BP2 | 11–77 | 78.9 | 81.9 | 0.57 | 90.3 | 87.1 | 0.03 | 0.60 |
| 65 | 1BP2 | 27–123 | −65.0 | −77.0 | 0.19 | −90.3 | −86.6 | 0.04 | 0.23 |
| 66 | 1BP2 | 29–45 | −66.9 | 151.9 | 1.23 | 97.6 | 95.5 | 0.02 | 1.25 |
| 67 | 1BP2 | 44–105 | −68.5 | −89.9 | 0.73 | −78.6 | −87.1 | 0.22 | 0.95 |
| 68 | 1BP2 | 51–98 | −93.2 | −60.0 | 1.04 | −79.0 | −87.0 | 0.19 | 1.23 |
| 69 | 1BP2 | 61–91 | −54.1 | 71.7 | 1.17 | 108.4 | 97.8 | 0.40 | 1.57 |
| 70 | 1BP2 | 84–96 | −76.0 | −92.0 | 0.98 | −96.8 | −87.1 | 0.25 | 1.23 |
| 71 | 2SGA | 42–58 | −146.7 | −107.5 | 2.76 | −87.3 | −88.1 | 0.00 | 2.76 |
| 72 | 2SGA | 191–220 | 95.0 | −54.8 | 1.96 | 101.4 | 96.9 | 0.08 | 2.04 |
| 73 | 3RP2 | A42–A58 | −153.3 | −86.2 | 1.49 | −88.2 | −87.0 | 0.00 | 1.49 |
| 74 | 3RP2 | A136–A201 | −125.8 | −79.6 | 2.43 | 113.3 | 94.7 | 1.11 | 3.54 |
| 75 | 3RP2 | A168–A182 | 82.4 | −50.1 | 1.72 | 158.9 | 97.4 | 5.37 | 7.09 |
| 76 | 3RP2 | B42–B58 | −151.5 | −86.8 | 1.62 | −80.5 | −87.1 | 0.13 | 1.75 |
| 77 | 3RP2 | B136–B201 | −140.0 | −68.8 | 2.00 | 98.9 | 95.6 | 0.05 | 2.05 |
| 78 | 3RP2 | B168–B182 | 76.7 | 48.1 | 0.98 | 116.0 | 85.5 | 2.01 | 2.99 |
| 79 | 2APP | 249–283 | −75.7 | 77.1 | 0.88 | 103.8 | 97.7 | 0.14 | 1.02 |
| 80 | 5RSA | 26–84 | −87.1 | −50.8 | 1.22 | −81.4 | −86.4 | 0.07 | 1.29 |
| 81 | 5RSA | 40–95 | −52.9 | −66.4 | 0.50 | −79.6 | −84.7 | 0.07 | 0.57 |
| 82 | 5RSA | 58–110 | −68.1 | −125.2 | 1.76 | −86.4 | −87.2 | 0.00 | 1.76 |
| 83 | 5RSA | 65–72 | −59.1 | 88.9 | 1.50 | 107.8 | 97.9 | 0.36 | 1.86 |
| 84 | 1TPP | 22–157 | −77.6 | 61.1 | 0.79 | 109.4 | 96.1 | 0.61 | 1.40 |
| 85 | 1TPP | 42–58 | −139.5 | −82.5 | 1.91 | −77.3 | −87.6 | 0.33 | 2.24 |
| 86 | 1TPP | 128–232 | −69.4 | 140.0 | 1.61 | 99.5 | 94.4 | 0.10 | 1.71 |
| 87 | 1TPP | 136–201 | −101.8 | −95.3 | 3.17 | 100.5 | 98.3 | 0.02 | 3.19 |
| 88 | 1TPP | 168–182 | 63.8 | 72.0 | 0.10 | 68.7 | 86.2 | 0.98 | 1.08 |
| 89 | 1TPP | 191–220 | 47.7 | 179.1 | 1.00 | 83.3 | 85.1 | 0.01 | 1.01 |
| 90 | 5PTI | 5–55 | −75.5 | −66.0 | 0.14 | −82.8 | −86.6 | 0.04 | 0.18 |
| 91 | 5PTI | 14–38 | 105.9 | −114.3 | 3.47 | 95.1 | 89.8 | 0.08 | 3.55 |
| 92 | 5PTI | 30–51 | −102.8 | −95.9 | 2.55 | −89.9 | −87.9 | 0.01 | 2.56 |

[a] 1ACX: actinoxanthin; 2AZA: azurin; 1REI, 2RHE: Bence–Jones protein; 5CPA: carboxypeptidase; 4CHA: α-chymotrypsin; 2CGA: chymotrypsinogen; 1CRN: crambin; 3EBX: erabutoxin; 3GRS: glutathione reductase; 3FAB: immunoglobulin; 3INS: insulin; 1LZ1: lysozyme; 2ALP: lytic protease; 1SN3, 1NXB: neurotoxin; 2OVO: ovomucoid; 9PAP: papain; 1BP2: phospholipase A2; 2SGA, 3RP2, 2APP: proteinase; 5RSA: ribonuclease; 1TPP: trypsin complex; 5PTI: trypsin inhibitor.

[b] Dihedral angle, in degrees. $\chi_1^2 = CC_1SS$, $\chi_2^2 = SSC_2C$, $\chi^3 = C_1SSC_2$, $\chi_0^3$ = ab initio minimum.

[c] Energy, in kcal/mole.

and $E(\Delta\chi^3) = 0.07$ for no. 14 with $\chi_0^3 = 97.4°$ and $E(\Delta\chi^3) = 5.37$ for no. 75]. (4) Although there are as many cases of $\chi_0^3 > 0$ (with an average value of 92.8°) as $\chi_0^3 < 0$ (with an average value of −86.7°), in both cases there is a preponderance of $|\Delta\chi^3| > 0$. These characteristics undoubtedly reflect the special nature of the surroundings of the disulfide bridge in a protein as compared to the simpler diethyl disulfide system.

It is useful to compare the results given by our ab initio energies with those predicted by the torsional potentials of present molecular mechanics functions. In Table IV we show such a comparison for some classes of observed left-handed and right-handed SS bridges,[4] for two engineered SS bridges in subtilisin,[4] and for the SS bridges of rat mast cell protease.[8] In the case of the left-handed bridges, the energies are similarly ordered but differ in relative

**Table IV Comparison Between Molecular Mechanics and Ab Initio Energies of Observed Disulfide Bridges**

| Protein | $\chi_1^{2a}$ | $\chi^{3a}$ | $\chi_2^{2a}$ | $\chi_0^{3a}$ | $E_{MM}(\chi^2, \chi^3)^b$ | $E_{AI}(\chi^2, \chi^3)^b$ |
|---|---|---|---|---|---|---|
| L.H.SS[c] | | | | | | |
| 1 | −57 | −85 | −64 | −84.7 | 0.53 | 0.31 |
| 2 | 173 | −84 | −171 | −86.8 | 0.67 | 0.61 |
| 3 | 171 | −82 | −171 | −86.8 | 0.71 | 0.72 |
| 4 | −65 | −80 | −93 | −87.0 | 1.70 | 1.07 |
| 5 | −87 | −89 | −148 | −87.3 | 2.52 | 1.79 |
| R.H.SS[d] | | | | | | |
| 1 | −61 | 98 | 63 | 97.6 | 0.99 | 0.87 |
| 2 | −56 | 103 | 74 | 98.1 | 1.61 | 1.21 |
| 3 | −78 | 103 | −166 | 97.5 | 1.96 | 1.45 |
| 4 | 170 | 92 | 43 | 84.9 | 1.20 | 1.53 |
| 5 | −71 | 103 | 84 | 98.0 | 2.18 | 1.20 |
| 6 | −83 | 101 | −121 | 95.0 | 3.82 | 2.76 |
| Subtilisin[e] | | | | | | |
| Cys 22–Cys 87 | 121 | −98 | 143 | −88.5 | 4.33 | 3.90 |
| Cys 24–Cys 87 | −50 | 96 | −171 | 97.1 | 1.11 | 1.75 |
| Protease[f] | | | | | | |
| Cys 42–Cys 58 | −153.3 | −88.2 | −86.2 | −87.6 | 1.94 | 2.73 |
| Cys 136–Cys 201 | −125.8 | 113.2 | −79.6 | −86.7 | 0.89 | 1.24 |
| Cys 168–Cys 182 | 82.4 | 158.9 | −50.1 | 86.1 | 1.46 | 2.32 |
| Cys 42–Cys 58 | −151.5 | −80.5 | −86.8 | 97.0 | 1.38 | 2.38 |
| Cys 136–Cys 201 | −140.0 | 98.9 | −68.8 | 86.4 | 1.70 | 2.18 |
| Cys 168–Cys 182 | 76.9 | 116.0 | 48.1 | 95.4 | 2.35 | 4.08 |

[a] Dihedral angle, in degrees. $\chi_1^2$ = $CC_1SS$, $\chi^3$ = $C_1SSC_2$, $\chi_2^2$ = $SSC_2C$, $\chi_0^3$ = ab initio minimum.
[b] Energy, in kcal/mole. MM: molecular mechanics (AMBER for L.H.SS, R.H.SS, and subtilisin, GROMOS for protease), AI: = ab initio.
[c] From Ref. 4. Numbers refer to classes of proteins with left-handed disulfides.
[d] From Ref. 4. Numbers refer to classes of proteins with right-handed disulfides.
[e] From Ref. 4. Refined x-ray structures of engineered disulfides.
[f] From Ref. 8.

and absolute magnitudes. In the case of the right-handed bridges, the order is significantly inverted in some cases and the magnitudes are substantially different. The subtilisin energies are in the same order but the magnitudes are significantly different. For the protease, the order of energies is again very different and the magnitudes are often widely apart. We think this reflects the oversimplified torsion potentials being assumed in the molecular mechanics functions for what is probably a coupled bridge system. Molecular mechanics energy functions usually assume that the potential function for $\chi^2$ in the SS bridge only has cos terms and is independent for $\chi_1^2$ and $\chi_2^2$. From Table AI we can see that the sin term coefficients $F_{10}^{sc} = -0.35$, $F_{20}^{sc} = -0.14$, and $F_{30}^{sc} = -0.13$ are all of the same order as the second cos term coefficient $F_{02}^{cc} = 0.54$, and therefore the assumption of an even potential function may not be valid in this case. As to the independence assumption, the cross-term coefficients $F_{11}^{sc} = -0.16$, $F_{11}^{ss} = 0.22$, and $F_{12}^{ss} = 0.13$ are also of the same order as $F^{cc}$. It is not likely that such terms can be deleted by separating the nonbonded interactions from the potential function and optimizing them.

## CONCLUSIONS

The ab initio energies of diethyl disulfide should provide a more accurate description of the energetics of the $\chi_1^2\chi^3\chi_2^2$ portion of the disulfide bridge in proteins than is obtainable from present molecular mechanics functions. Differences are certainly seen between relative energies calculated by these two methods for observed or predicted bridges, and these may be due to the limited nature of the torsion potentials used in the energy functions. For small departures from $\chi_0^3$ ($|\Delta\chi^3| < 20°$), the energy depends primarily on $\chi_1^2$ and $\chi_2^2$, with a relatively constant increment for $\Delta\chi^3$ whose value depends only on whether $\chi_0^3$ is smaller or larger than 90°. This information plus the $E(\chi_1^2, \chi_2^2)$ map makes it possible to readily determine the relevant energy of a disulfide bridge once $\chi_1^2$, $\chi_2^2$, and $\chi^3$ are given.

The ab initio studies also show that $\chi_0^3$ is quite variable, having values between < 83° and > 114°. Nor are departures from the widely assumed equilibrium value of ~ 90° attributable to higher energies of the bridge, presumably from nonbonded interactions. These studies therefore suggest that the total electronic structure must be taken into account in order to properly describe the energies and structures of the disulfide bridge.

## APPENDIX

Since the energy $E$ is a periodic function of $\chi_1^2$ and $\chi_2^2$, it can be represented by a truncated two-dimensional Fourier series on the assumption that the highest frequency component is not larger than $N/2$, viz.,

$$E(\chi_1^2, \chi_2^2) = \sum_{m=0}^{N-1} \sum_{n=0}^{N-1} F_{mn} \times \exp[-i\tau(m\chi_1^2 + n\chi_2^2)] \quad \text{(A1)}$$

**Table AI Coefficients of Fourier Series for $E(\chi_1^2, \chi_2^2)$ [Eq. (A4)]**

$F_{mn}^{cc}$

| m \ n | 0 | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|
| 0 | 3.0147 | 0.847800 | 0.536770 | 1.0409 | 0.013197 | −0.054787 | −0.041513 |
| 1 | | 0.057098 | −0.079923 | −0.076815 | 0.017264 | 0.016289 | −0.006821 |
| 2 | | | −0.034413 | −0.003889 | −0.013304 | −0.016798 | 0.003849 |
| 3 | | | | 0.043571 | −0.020427 | −0.001367 | 0.002678 |
| 4 | | | | | 0.000529 | 0.010502 | −0.008309 |
| 5 | | | | | | −0.011344 | −0.004365 |
| 6 | | | | | | | 0.006409 |

$F_{mn}^{sc}$

| m \ n | 0 | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|
| 1 | −0.346550 | −0.161950 | 0.045841 | 0.077266 | −0.037407 | 0.006122 | 0.010101 |
| 2 | −0.144100 | −0.052890 | 0.059914 | 0.061352 | −0.023006 | 0.012132 | 0.011711 |
| 3 | −0.131100 | −0.029800 | −0.003000 | −0.021453 | 0.020863 | 0.019568 | 0.006887 |
| 4 | 0.041358 | −0.024509 | −0.004729 | 0.010313 | 0.008968 | −0.009079 | −0.001200 |
| 5 | −0.016032 | −0.021542 | 0.015050 | 0.023329 | −0.013131 | 0.005318 | 0.003513 |

$F_{mn}^{ss}$

| m \ n | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| 1 | 0.22204 | 0.127310 | 0.033926 | 0.020998 | 0.037757 |
| 2 | | 0.053418 | 0.007215 | 0.024386 | 0.013913 |
| 3 | | | −0.031389 | 0.022615 | 0.008852 |
| 4 | | | | 0.007189 | −0.005376 |
| 5 | | | | | 0.021852 |

where the sampling interval $\tau$ is $360°/N$ and the coefficients $F_{mn}$ are given by the discrete Fourier transformation

$$F_{mn} = \frac{1}{N^2} \sum_{\chi_1^2=0}^{N-1} \sum_{\chi_2^2=0}^{N-1} E(\chi_1^2, \chi_2^2) \exp[i\tau(m\chi_1^2 + n\chi_2^2)]$$

$$\times\, m, n = 0, 1, \ldots, N-1 \quad \text{(A2)}$$

By substituting (A2) into (A1), expanding the exponentials, and making use of the symmetry and orthogonality properties of the various summed products, we find that

$$E(\chi_1^2, \chi_2^2) = \frac{1}{N^2} \sum_{m=0}^{N-1} \sum_{n=0}^{N-1} [F_{mn}^{cc} \cos m\chi_1^2 \cos n\chi_2^2 + F_{mn}^{ss} \sin m\chi_1^2 \sin n\chi_2^2 + F_{mn}^{cs} \cos m\chi_1^2 \sin n\chi_2^2 + F_{mn}^{sc} \sin m\chi_1^2 \cos n\chi_2^2] \quad \text{(A3)}$$

with

$$F_{mn}^{cc} = \sum_{\chi_1^2=0}^{N-1} \sum_{\chi_2^2=0}^{N-1} \cos m\chi_1^2 \cos n\chi_2^2$$

$$F_{mn}^{ss} = \sum_{\chi_1^2=0}^{N-1} \sum_{\chi_2^2=0}^{N-1} \sin m\chi_1^2 \sin n\chi_2^2$$

**Table AII Coefficients of Fourier Series for $\chi_0^3(\chi_1^2, \chi_2^2)$ [Eq. (A4)]**

| | $F_{mn}^{cc}$ | | | | | | |
|---|---|---|---|---|---|---|---|
| | $n$ | | | | | | |
| $m$ | 0 | 1 | 2 | 3 | 4 | 5 | 6 |
| 0 | 91.683 | 2.2608 | −1.43410 | −0.896220 | 0.473180 | 0.376160 | 0.109440 |
| 1 | | 0.2071 | −0.69768 | −0.414550 | 0.305280 | −0.179860 | −0.083668 |
| 2 | | | −0.32891 | 0.079237 | −0.274650 | −0.182360 | 0.072627 |
| 3 | | | | 0.541920 | −0.294700 | −0.167560 | 0.000847 |
| 4 | | | | | 0.054598 | −0.083145 | 0.074219 |
| 5 | | | | | | −0.111820 | −0.058893 |
| 6 | | | | | | | 0.006335 |

| | $F_{mn}^{sc}$ | | | | | | |
|---|---|---|---|---|---|---|---|
| | $n$ | | | | | | |
| $m$ | 0 | 1 | 2 | 3 | 4 | 5 | 6 |
| 1 | −4.07290 | −1.22270 | 0.21973 | 0.484040 | −0.541730 | −0.006197 | 0.149150 |
| 2 | −2.82940 | −0.12894 | 0.40904 | 0.473650 | −0.167980 | 0.090300 | 0.070256 |
| 3 | −0.31754 | 0.14594 | 0.30129 | 0.162440 | −0.011696 | 0.147460 | 0.072773 |
| 4 | 0.34121 | −0.40549 | −0.26651 | −0.077994 | −0.051936 | −0.149070 | −0.057131 |
| 5 | −0.15415 | −0.10019 | 0.15957 | 0.152660 | −0.138570 | −0.042011 | 0.047482 |

| | $F_{mn}^{ss}$ | | | | |
|---|---|---|---|---|---|
| | $n$ | | | | |
| $m$ | 1 | 2 | 3 | 4 | 5 |
| 1 | 1.4532 | 0.68126 | 0.210030 | 0.15042 | 0.283370 |
| 2 | | 0.12587 | 0.037071 | 0.28062 | 0.182260 |
| 3 | | | −0.267780 | 0.23380 | 0.143020 |
| 4 | | | | −0.13210 | −0.097876 |
| 5 | | | | | 0.123440 |

$$F^{cs}_{mn} = \sum_{\chi_1^2=0}^{N-1} \sum_{\chi_2^2=0}^{N-1} \cos m\chi_1^2 \sin n\chi_2^2$$

$$F^{sc}_{mn} = \sum_{\chi_1^2=0}^{N-1} \sum_{\chi_2^2=0}^{N-1} \sin m\chi_1^2 \cos n\chi_2^2$$

where the units of $\chi_1^2$ and $\chi_2^2$ are taken to be $\tau$.

For a $\chi_1^2$, $\chi_2^2$ symmetric function, Eq. (A3) has the more explicit form

$$\begin{aligned}
E(\chi_1^2, \chi_2^2) = F^{cc}_{00} + \sum_{m=1}^{6} [F^{cc}_{0m}(\cos m\chi_1^2 + \cos m\chi_2^2) \\
+ F^{cc}_{mm}\cos m\chi_1^2 \cos m\chi_2^2 \\
+ \sum_{n=m+1}^{6} F^{cc}_{mn}(\cos m\chi_1^2 \cos n\chi_2^2 \\
+ \cos n\chi_1^2 \cos m\chi_2^2)] \\
+ \sum_{m=1}^{5} [F^{ss}_{mm}\sin m\chi_1^2 \sin m\chi_2^2 \\
+ \sum_{n=m+1}^{5} F^{ss}_{mn}(\sin m\chi_1^2 \sin n\chi_2^2 \\
+ \sin n\chi_1^2 \sin m\chi_2^2)] \\
+ \sum_{m=1}^{5} [F^{sc}_{m0}(\sin m\chi_1^2 + \sin m\chi_2^2) \\
+ \sum_{n=1}^{6} F^{sc}_{mn}(\sin m\chi_1^2 \cos n\chi_2^2 \\
+ \cos n\chi_1^2 \sin m\chi_2^2)] \quad \text{(A4)}
\end{aligned}$$

where the coefficients are given by

$$F^{cc}_{mn} = \sum_{\chi_1^2=0}^{11} \sum_{\chi_2^2=0}^{11} E(\chi_1^2, \chi_2^2)\cos m\chi_1^2 \cos n\chi_2^2 \times m, n \geq m = 0, 1, \ldots, 6 \quad \text{(A5)}$$

$$F^{ss}_{mn} = \sum_{\chi_1^2=0}^{11} \sum_{\chi_2^2=0}^{11} E(\chi_1^2, \chi_2^2)\sin m\chi_1^2 \sin n\chi_2^2 \times m, n \geq m = 1, 2, \ldots, 5 \quad \text{(A6)}$$

$$F^{sc}_{mn} = \sum_{\chi_1^2=0}^{11} \sum_{\chi_2^2=0}^{11} E(\chi_1^2, \chi_2^2)\sin m\chi_1^2 \cos n\chi_2^2 \times m = 1, 2, \ldots, 5; n = 0, 1, \ldots, 6 \quad \text{(A7)}$$

In Eq. (A4), the normalization of the $F_{mn}$ has been taken into account: the $F^{cc}_{00}$, $F^{cc}_{06}$, and $F^{cc}_{66}$ were divided by 144; the $F^{cc}_{mn}$, $F^{ss}_{mn}$, and $F^{sc}_{mn}$ ($m, n = 1, 2, \ldots, 5$) were divided by 36; and the $F^{cc}_{0n}$, $F^{cc}_{m6}$, $F^{sc}_{m6}$, and $F^{sc}_{m0}$ ($m, n = 1, 2, \ldots, 5$) were divided by 72.

The transformations (A5)-(A7) require that the data be sampled evenly, but the $E(\chi_1^2, \chi_2^2)$ for the stationary state conformers do not occur at the exact 30° intervals (see Table I). It was therefore necessary to refine the $F_{mn}$, which was done using a least-squares fitting program. The final coefficients reproduced the 78 $E(\chi_1^2, \chi_2^2)$ to the order of $10^{-4}$ kcal/mole. These coefficients are given in Table AI.

A Fourier series identical to that in Eq. (A4) is appropriate for representing $\chi_0^3(\chi_1^2, \chi_2^2)$, and the coefficients for this series are given in Table AII.

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