Solid State and Solution Conformation of Phenylacetyl-L-Cysteinyl-D-penicillamine Cyclic Disulphide Methyl Ester; a Cyclic Dipeptide containing a *trans* Amide

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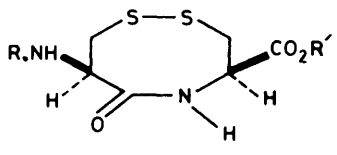
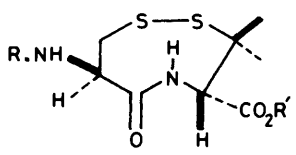
The conformation of the title compound (**2a**) has been determined by *X*-ray crystallography and by solution ^{1}H n.m.r. studies. The compound crystallises in the triclinic space group *P*1 and its structure was solved and refined to an *R* factor of 0.051, from 1 256 observed reflections. In the solid state the molecules possess a distorted *trans* lactam structure ($\Delta\omega \approx 30°$) with a P-helical disulphide bridge. Individual molecules in the crystal are H-bonded between the side chain amide and lactam functions to form a β-pleated sheet array. Assignment of the solution conformation was carried out by comparison of coupling constants with calculated values derived from crystal data, comparison of chemical shifts, and temperature coefficients of the NH resonances in $(CD_3)_2SO$ and $CDCl_3$ and by n.O.e. difference measurements. The structure and conformation of (**2a**) in solution was found to be similar to that in the solid state. The sulphone (**6**) was shown to have a similar solution conformation.

Ring systems containing both amide groups and disulphide bridges are important structural elements in a wide range of biologically active compounds. These range from the rigid cysteine diketopiperazine system found in gliotoxin[1] and the antiviral agent, aranotin,[2] to the macrocyclic and conformationally more flexible disulphide bridged structures present in the pentapeptide malformin A[3] and the cyclodepsipeptide quinoxaline antibiotics.[4] Medium-sized ring compounds possessing lactam and disulphide functions are uncommon however, and few reports of their structures and conformations



(**1**) R = R′ = H

(**1a**) R = Bu^tO_2C, R′ = Me



(**2**) R = Acyl, R′ = Alkyl or H

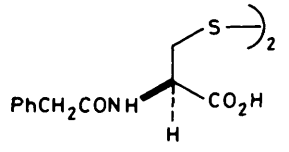
(**2a**) R = $PhCH_2CO$, R′ = Me

are available. Of these L-cysteinyl-L-cysteine cyclic disulphide (**1**),[5] which possesses the 6-oxoperhydro-1,2,5-dithiazocine ring system, has received most attention. Crystallographic studies of (**1**) and its t-butyloxycarbonyl methyl ester (**1a**) have shown that the compounds have a distorted *cis* lactam function and that the disulphide bridge adopts a right-handed helical (P-helical) conformation.[6,7]

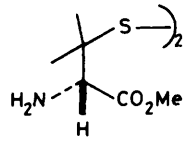
In the course of studies on the formation of penicillin ring structures we needed to synthesize compounds of the general structure (**2**; R = acyl). We discuss here the solid state and solution conformation of the cyclic disulphide (**2a**), which possesses an endocyclic *trans* amide group.

Discussion

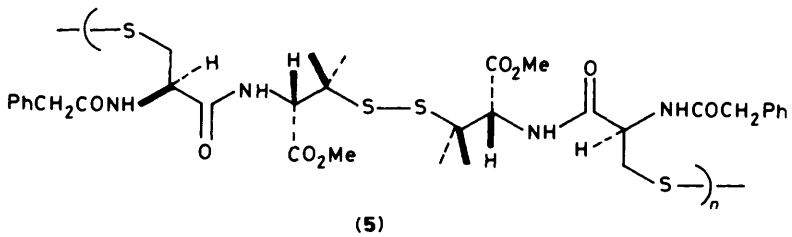
The synthesis of (**2a**) was carried out in a straightforward manner by coupling of *N*,*N*′-bisphenylacetyl-L-cystine (**3**) with bis-D-penicillamine methyl ester (**4**) using *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) in tetrahydrofuran. The polymeric disulphide linked product (**5**) was reduced with zinc–HCl to the free dithiol which was oxidised with iodine to afford (**2a**). Solution and refinement of the structure is described in the Experimental section. There are two crystallographically unrelated but virtually identical molecules in the unit cell. A stereoscopic diagram showing atomic labelling for one of these is shown in Figure 1; for the other molecule the same numbering scheme with primes is used. Final fractional co-ordinates are given in Table 1 for the non-hydrogen atoms. Derived bond lengths, valence angles and torsion angles are given in Tables 2, 3, and 4. The only significant difference



(**3**)



(**4**)



(**5**)

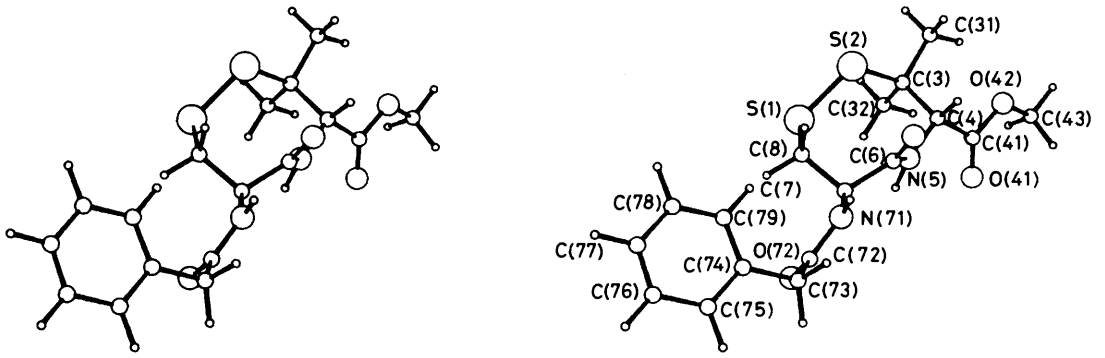


Figure 1. Stereoscopic diagram of one molecule of (**2a**), showing numbering scheme used

Table 1. Fractional co-ordinates for compound (**2a**) with standard deviations

	x	*y*	*z*
S(1)	0.405 5(6)	0.106 4(6)	0.259 9(5)
S(2)	0.521 40	0.275 80	0.170 70
C(3)	0.719 2(16)	0.272 3(16)	0.160 5(13)
C(4)	0.830 2(16)	0.354 8(16)	0.284 5(13)
N(5)	0.757 7(12)	0.289 8(12)	0.382 5(10)
C(6)	0.675 2(16)	0.350 6(16)	0.447 2(13)
C(7)	0.551 4(14)	0.237 9(14)	0.499 6(12)
C(8)	0.400 0(16)	0.188 4(16)	0.408 8(13)
C(31)	0.771 5(19)	0.367 0(17)	0.058 0(14)
C(32)	0.716 2(18)	0.113 8(16)	0.132 8(14)
C(41)	0.988 0(17)	0.329 0(17)	0.284 9(15)
O(41)	1.029 0(12)	0.256 7(12)	0.349 3(10)
O(42)	1.066 7(11)	0.404 1(11)	0.203 3(10)
C(43)	1.203 4(16)	0.373 9(16)	0.186 6(14)
O(61)	0.687 2(12)	0.484 4(11)	0.451 0(9)
N(71)	0.523 4(14)	0.300 3(13)	0.612 8(11)
C(72)	0.460 1(16)	0.208 3(18)	0.691 6(13)
O(72)	0.446 6(13)	0.079 8(11)	0.679 6(11)
C(73)	0.402 6(17)	0.279 2(17)	0.797 7(14)
C(74)	0.228 6(15)	0.228 4(15)	0.777 0(12)
C(75)	0.146 2(19)	0.177 9(17)	0.870 3(16)
C(76)	−0.019 6(20)	0.129 3(18)	0.851 7(17)
C(77)	−0.092 3(20)	0.136 0(17)	0.745 7(15)
C(78)	−0.011 6(22)	0.183 0(20)	0.652 1(18)
C(79)	0.145 6(18)	0.231 6(16)	0.669 4(14)
S(1′)	0.187 2(7)	0.736 8(7)	0.465 9(5)
S(2′)	0.085 3(6)	0.575 6(6)	0.568 1(5)
C(3′)	0.108 6(17)	0.663 4(16)	0.725 8(14)
C(4′)	0.269 5(15)	0.686 0(15)	0.788 4(12)
N(5′)	0.386 2(15)	0.773 2(12)	0.724 0(13)
(C6′)	0.464 1(15)	0.709 1(16)	0.654 5(12)
C(7′)	0.506 9(14)	0.787 8(14)	0.546 1(11)
C(8′)	0.371 5(16)	0.718 6(16)	0.443 9(13)
C(31′)	−0.018 9(17)	0.547 6(17)	0.776 5(14)
C(32′)	0.074 3(17)	0.809 7(15)	0.726 4(13)
C(41′)	0.298 7(18)	0.765 1(20)	0.916 3(16)
O(41′)	0.356 9(14)	0.898 6(11)	0.942 8(10)
O(42′)	0.242 6(13)	0.672 3(12)	0.993 1(9)
C(43′)	0.259 6(19)	0.742 1(18)	1.115 4(15)
O(61′)	0.479 8(12)	0.591 8(10)	0.667 2(9)
N(71′)	0.644 5(14)	0.770 9(14)	0.504 7(11)
C(72′)	0.726 8(18)	0.868 9(18)	0.438 3(14)
O(72′)	0.700 0(12)	0.981 1(11)	0.420 6(10)
C(73′)	0.855 0(16)	0.830 4(17)	0.384 2(13)
C(74′)	0.818 5(17)	0.801 7(16)	0.250 7(14)
C(75′)	0.937 4(21)	0.868 8(19)	0.182 8(17)
C(76′)	0.904(3)	0.845 5(22)	0.058 9(19)
C(77′)	0.767 3(23)	0.757 1(20)	0.004 6(19)
C(78′)	0.648 3(25)	0.688 7(23)	0.065 9(18)
C(79′)	0.675 2(19)	0.705 7(18)	0.192 3(16)

Table 2. Bond lengths (Å) for compound (**2a**) with standard deviations

S(1)–S(2)	2.057(6)	S(1′)–S(2′)	2.066(8)
S(1)–C(8)	1.814(16)	S(1′)–C(8′)	1.823(16)
S(2)–C(3)	1.861(15)	S(2′)–C(3′)	1.855(16)
C(3)–C(4)	1.565(21)	C(3′)–C(4′)	1.477(21)
C(3)–C(31)	1.569(23)	C(3′)–C(31′)	1.539(22)
C(3)–C(32)	1.523(22)	C(3′)–C(32′)	1.550(21)
C(4)–N(5)	1.459(19)	C(4′)–N(5′)	1.454(19)
C(4)–C(41)	1.561(22)	C(4′)–C(41′)	1.515(23)
N(5)–C(6)	1.367(19)	N(5′)–C(6′)	1.386(19)
C(6)–C(7)	1.516(20)	C(6′)–C(7′)	1.529(19)
C(6)–O(61)	1.252(18)	C(6′)–C(61′)	1.207(18)
C(7)–C(8)	1.500(20)	C(7′)–C(8′)	1.479(20)
C(7)–N(71)	1.465(19)	C(7′)–N(71′)	1.475(18)
C(41)–O(41)	1.161(20)	C(41′)–O(41′)	1.199(21)
C(41)–O(42)	1.364(19)	C(41′)–O(42′)	1.332(21)
O(42)–C(43)	1.428(19)	O(42′)–C(43′)	1.446(21)
N(71)–C(72)	1.365(20)	N(71′)–C(72′)	1.354(21)
C(72)–O(72)	1.195(19)	C(72′)–O(72′)	1.217(20)
C(72)–C(73)	1.580(23)	C(72′)–C(73′)	1.551(22)
C(73)–C(74)	1.482(21)	C(73′)–C(74′)	1.477(22)
C(74)–C(75)	1.418(22)	C(74′)–C(75′)	1.437(25)
C(74)–C(79)	1.345(21)	C(74′)–C(79′)	1.349(24)
C(75)–C(76)	1.41(3)	C(75′)–C(76′)	1.37(3)
C(76)–C(77)	1.30(3)	C(76′)–C(77′)	1.27(3)
C(77)–C(79)	1.40(3)	C(77′)–C(78′)	1.39(3)
C(78)–C(79)	1.34(3)	C(78′)–C(79′)	1.40(3)

between the two molecules is in the methoxycarbonyl group, where differences in the torsion angles about the C(4)–C(41) bond may be seen.

Solid State Conformation.—The two independent molecules in the unit cell are arranged in a head-to-tail manner. Each molecule is linked by intermolecular H-bonds between the amide of the phenylacetylamino substituent and the lactam groups of its neighbours in an arrangement analogous to the β-pleated sheet structures of larger polypeptides (Figure 2). The principal feature of the molecular architecture is the presence of a distorted *trans* amide linkage in the ring with torsion angles C(4)–N(5)–C(5)–C(7) (ω) of 152.9 and 148.4°. The effect of ring size on endocyclic amide deformation has been studied previously. In simple lactams the crossover from *trans* to *cis* stereochemistry has been found to occur with the nine-membered ring compound, caprylolactam, which exists as a *trans* amide in the solid state,[8] and as a 4:1 mixture of *cis* and *trans* forms in solution.[9] The *trans* amide of caprylolactam is also distorted in the solid state with ω = 148.4°.

On the basis of energy calculations two theoretical models for the conformations of the eight-membered ring of L-cysteinyl-L-cysteine cyclic disulphide (**1**) have been advanced.[10] The

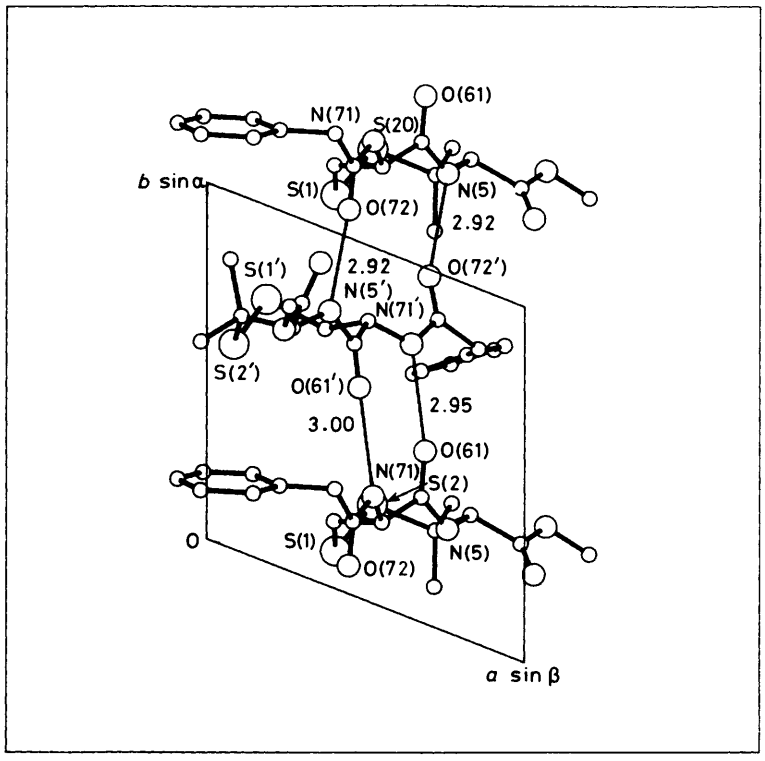


Figure 2. Projection of compound (**2a**) along *c*, showing hydrogen bonding with N · · · O contact distances given in Å

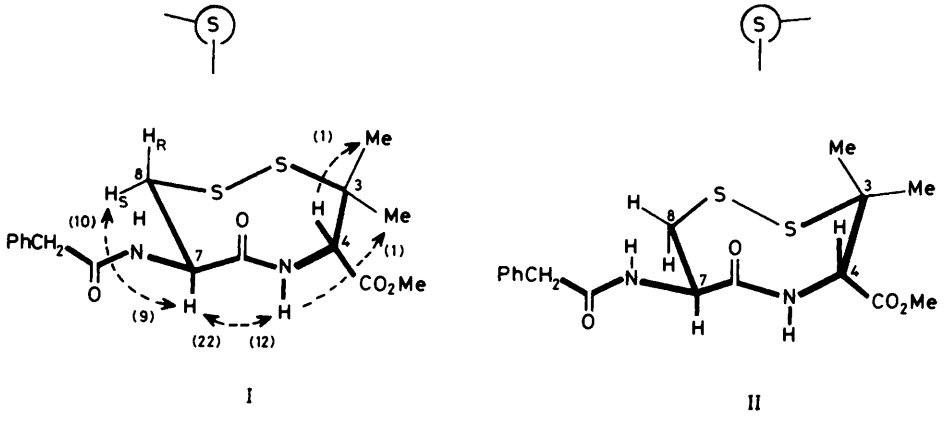


Figure 3. P-Helical (I) and M-helical (II) conformations for (**2a**). The perspectives viewed along the S–S bonds of each conformation are shown above the formulae. The principal n.O.e.s observed in the ^{1}H n.m.r. spectrum are indicated on conformer I. Figures in parentheses are the % enhancement values measured in the difference n.O.e. experiment (8 mM solution in $CDCl_3$ at 25 °C)

minimum energy form has an amide distortion ($\Delta\omega$) of $-12°$ and a left-handed (M-helical) disulphide conformation with a CSSC torsion angle of 98°. The higher energy form has an amide distortion of $+14°$ and a right-handed (P-helical) disulphide conformation with a CSSC torsion angle of 100°. The crystal structures of (**1**) and (**1a**) show, however, that these possess $\Delta\omega$ values of -7.2 and $+10.8°$, respectively, and that both have P-helical disulphide conformations with CSSC angles of 94 and 96° respectively.[6,7] In (**2a**) the disulphide bridge also has a P-helical conformation with C(8)–S(1)–S(2)–C(3) torsion angles of 107.7 and 105.0° in the two molecules. The amide distortion [180°–C(4)–N(S)–C(6)–C(7)] is -27.1 and $-31.6°$.

Consideration of molecular models of the cyclic peptides (**1**) and (**1a**) indicate that inversion of the stereochemistry at C-4 from L- to D- would result in significant non-bonded interactions between the carboxylate (or methoxycarbonyl) substituent and the *pro* R hydrogen at C-8. Minimisation of the transannular interactions in (**2a**) can thus be achieved in a distorted *trans* lactam which allows the methoxycarbonyl substituent to adopt an equatorial orientation.

Solution Conformation.—From i.r. and molecular polarisation evidence caprylolactam has been shown to exist as an equilibrium mixture of *cis* and *trans* lactams in solution.[9] The solution conformation of (**1**) has previously been examined by comparing experimental ^{1}H n.m.r. chemical shifts and coupling constants with values predicted from the crystal structure.[11] On this basis (**1**) appears to exist solely as the *cis* form in solution

Table 3. Angles (°) for compound (**2a**) with standard deviations

S(2)–C(1)–C(8)	108.5(5)	S(2′)–S(1′)–C(8′)	107.2(6)
S(1)–S(2)–C(3)	110.0(5)	S(1′)–S(2′)–C(3′)	110.1(6)
S(2)–C(3)–C(4)	106.5(10)	S(2′)–C(3′)–C(4′)	106.9(10)
S(2)–C(3)–C(31)	104.9(10)	S(2′)–C(3′)–C(31′)	104.0(10)
S(2)–C(3)–C(32)	112.4(11)	S(2′)–C(3′)–C(32′)	110.2(10)
C(4)–C(3)–C(31)	109.4(12)	C(4′)–C(3′)–C(31′)	112.4(13)
C(4)–C(3)–C(32)	111.4(12)	C(4′)–C(3′)–C(32′)	113.7(12)
C(31)–C(3)–C(32)	111.9(13)	C(31′)–C(3′)–C(32′)	109.2(12)
C(3)–C(4)–N(5)	109.1(12)	C(3′)–C(4′)–N(5′)	110.9(12)
C(3)–C(4)–C(41)	106.7(12)	C(3′)–C(4′)–C(41′)	109.3(12)
N(5)–C(4)–C(41)	110.0(12)	N(5′)–C(4′)–C(41′)	110.0(12)
C(4)–N(5)–C(6)	123.9(12)	C(4′)–N(5′)–C(6′)	122.4(12)
N(5)–C(6)–C(7)	114.3(12)	N(5′)–C(6′)–C(7′)	113.5(12)
N(5)–C(6)–O(61)	123.0(13)	N(5′)–C(6′)–O(61′)	124.0(13)
C(7)–C(6)–O(61)	122.2(13)	C(7′)–C(6′)–O(61′)	121.8(13)
C(6)–C(7)–C(8)	107.0(11)	C(6′)–C(7′)–C(8′)	107.5(11)
C(6)–C(7)–N(71)	113.4(11)	C(6′)–C(7′)–N(71′)	114.1(11)
C(8)–C(7)–N(71)	107.2(11)	C(8′)–C(7′)–N(71′)	106.6(11)
S(1)–C(8)–C(7)	115.4(10)	S(1′)–C(8′)–C(7′)	116.0(11)
C(4)–C(41)–O(41)	122.3(15)	C(4′)–C(41′)–O(41′)	122.8(15)
C(4)–C(41)–O(42)	110.8(13)	C(4′)–C(41′)–O(42′)	113.1(14)
O(41)–C(41)–O(42)	126.9(15)	O(41′)–C(41′)–O(42′)	123.9(16)
C(41)–O(42)–C(43)	115.1(12)	C(41′)–O(42′)–C(43′)	115.7(13)
C(7)–N(71)–C(72)	120.2(12)	C(7′)–N(71′)–C(72′)	120.7(12)
N(71)–C(72)–O(72)	121.4(14)	N(71′)–C(72′)–O(72′)	121.5(15)
N(71)–C(72)–C(73)	116.8(13)	N(71′)–C(72′)–C(73′)	117.0(14)
O(72)–C(72)–C(73)	121.8(14)	O(72′)–C(72′)–C(73′)	121.5(14)
C(72)–C(73)–C(74)	111.8(13)	C(72′)–C(73′)–C(74′)	112.6(13)
C(73)–C(74)–C(75)	121.0(13)	C(73′)–C(74′)–C(75′)	120.4(14)
C(73)–C(74)–C(79)	120.5(14)	C(73′)–C(74′)–C(79′)	119.7(15)
C(75)–C(74)–C(79)	118.5(14)	C(75′)–C(74′)–C(79′)	119.8(15)
C(74)–C(75)–C(76)	121.6(15)	C(74′)–C(75′)–C(76′)	120.9(17)
C(75)–C(76)–C(77)	116.6(17)	C(75′)–C(76′)–C(77′)	118.7(20)
C(76)–C(77)–C(78)	122.1(17)	C(76′)–C(77′)–C(78′)	122.6(21)
C(77)–C(78)–C(79)	121.7(18)	C(77′)–C(78′)–C(79′)	121.5(19)
C(74)–C(79)–C(78)	119.4(16)	C(74′)–C(79′)–C(78′)	116.1(17)

although it is not known whether the disulphide bridge adopts the P- or M-helical conformation. In order to determine whether the solution conformation of (**2a**) is similar to that found in the solid state we have carried out a study of the former using ^{1}H n.m.r. techniques.

(1) *Proton Chemical Shifts and Coupling Constants.*—The chemical shifts of the proton resonances of (**2a**) in $CDCl_3$ and $(CD_3)_2SO$ and the coupling constants in $CDCl_3$ solution are shown in Table 5. In $(CD_3)_2SO$ the chemical shifts of both amide protons are shifted to higher frequencies than in the $CDCl_3$ spectrum as a result of H-bonding with the solvent, an observation consistent with neither proton being involved in intramolecular H-bonding.[12] As expected both NH protons show relatively large negative temperature coefficients in $(CD_3)_2SO$ (Table 6), since H-bonding to the oxygen atoms of the solvent is disrupted with increasing temperature.[12] In $CDCl_3$ the small temperature coefficient for the penicillaminyl NH indicates no H-bonding while the large negative coefficient for the cysteinyl NH suggests that this proton is involved in intermolecular H-bonding at lower temperatures. It is possible that in dilute $CDCl_3$ solution at low temperatures (**2a**) exists as H-bonded dimers.

Various treatments have been used to calculate vicinal coupling constants in the H–N–C_α–H system of peptides from torsion angles.[13–16] While absolute values differ with the methods of calculation employed, J_{trans} is always greater than J_{cis}. In the case of (**2a**) the value of $J_{\alpha,NH}$ for the penicillaminyl residue (11.1 Hz) is greater than that predicted by the majority of methods but approaches the value of 12.4 Hz calculated using the modified Karplus treatment of Barfield and Gearhart[16] for a torsion angle of 158°, indicating that the lactam function of (**2a**) has *trans* geometry. In contrast, the lower value for $J_{\alpha,NH}$ of the cysteine residue indicates that the *trans* orientation of 7-H and N-H found in the solid state is not maintained in solution. The value of 7.0 Hz can best be rationalised as reflecting an

Table 4. Torsion angles (°) for compound (**2a**) with standard deviations

C(8)–S(1)–S(2)–C(3)	107.7(7)	C(7)–N(71)–C(72)–O(72)	10.6(22)	N(5′)–C(4′)–C(41′)–O(41′)	29.7(22)
S(2)–S(1)–C(8)–C(7)	−76.2(11)	C(7)–N(71)–C(72)–C(73)	−168.2(12)	N(5′)–C(4′)–C(41′)–O(42′)	−155.0(13)
S(1)–S(2)–C(3)–C(4)	−81.8(10)	N(71)–C(72)–C(73)–C(74)	105.6(16)	C(4′)–N(5′)–C(6′)–C(7′)	148.4(13)
S(1)–S(2)–C(3)–C(31)	162.2(9)	O(72)–C(72)–C(73)–C(74)	−73.3(19)	C(4′)–N(5′)–C(6′)–O(61′)	−22.5(22)
S(1)–S(2)–C(3)–C(32)	40.4(12)	C(72)–C(73)–C(74)–C(75)	133.0(15)	N(5′)–C(6′)–C(7′)–C(8′)	−88.2(14)
S(2)–C(3)–C(4)–N(5)	51.8(13)	C(72)–C(73)–C(74)–C(79)	−48.1(19)	N(5′)–C(6′)–C(7′)–N(71′)	153.9(12)
S(2)–C(3)–C(4)–C(41)	170.7(10)	C(73)–C(74)–C(75)–C(76)	180.0(15)	O(61′)–C(6′)–C(7′)–C(8′)	82.9(16)
C(31)–C(3)–C(4)–N(5)	164.7(12)	C(79)–C(74)–C(75)–C(76)	1.1(24)	O(61′)–C(6′)–C(7′)–N(71′)	−35.0(19)
C(31)–C(3)–C(4)–C(41)	−76.4(15)	C(73)–C(74)–C(79)–C(78)	179.0(16)	C(6′)–C(7′)–C(8′)–S(1′)	62.0(14)
C(32)–C(3)–C(4)–N(5)	−71.1(15)	C(75)–C(74)–C(79)–C(78)	−2.2(24)	N(71′)–C(7′)–C(8′)–S(1′)	−175.3(10)
C(32)–C(3)–C(4)–C(41)	47.8(16)	C(74)–C(75)–C(76)–C(77)	−1.4(26)	C(6′)–C(7′)–C(71′)–C(72′)	−158.8(13)
C(3)–C(4)–N(5)–C(6)	−98.5(15)	C(75)–C(76)–C(77)–C(78)	2.7(27)	C(8′)–C(7′)–N(71′)–C(72′)	82.8(16)
C(41)–C(4)–N(5)–C(6)	144.7(13)	C(76)–C(77)–C(78)–C(79)	−3.9(30)	C(4′)–C(41′)–O(42′)–C(43′)	−177.0(13)
C(3)–C(4)–C(41)–O(41)	−122.3(17)	C(77)–C(78)–C(79)–C(74)	3.5(28)	O(41′)–C(41′)–O(42′)–C(43′)	−1.7(24)
C(3)–C(4)–C(41)–O(42)	68.9(15)	C(8′)–S(1′)–S(2′)–C(3′)	105.0(8)	C(7′)–N(71′)–C(72′)–O(72′)	7.1(23)
N(5)–C(4)–C(41)–O(41)	5.9(21)	S(2′)–S(1′)–C(8′)–C(7′)	−80.2(11)	C(7′)–N(71′)–C(72′)–C(73′)	−171.9(12)
N(5)–C(4)–C(41)–O(42)	−172.8(12)	S(1′)–S(2′)–C(3′)–C(4′)	−80.8(10)	N(71′)–C(72′)–C(73′)–C(74′)	115.1(16)
C(4)–N(5)–C(6)–C(7)	152.9(13)	S(1′)–S(2′)–C(3′)–C(31′)	160.1(9)	O(72′)–C(72′)–C(73′)–C(74′)	−64.0(20)
C(4)–N(5)–C(6)–O(61)	−19.1(22)	S(1′)–S(2′)–C(3′)–C(32′)	43.2(11)	C(72′)–C(73′)–C(74′)–C(75′)	133.7(16)
N(5)–C(6)–C(7)–C(8)	−92.1(14)	S(2′)–C(3′)–C(4′)–N(5′)	55.6(13)	C(72′)–C(73′)–C(74′)–C(79′)	−49.9(20)
N(5)–C(6)–C(7)–N(71)	149.8(12)	S(2′)–C(3′)–C(4′)–C(41′)	177.1(10)	C(73′)–C(74′)–C(75′)–C(76′)	−178.6(17)
O(61)–C(6)–C(7)–C(8)	79.9(17)	C(31′)–C(3′)–C(4′)–N(5′)	169.1(12)	C(79′)–C(74′)–C(75′)–C(76′)	4.9(27)
O(61)–C(6)–C(7)–N(71)	−38.2(19)	C(31′)–C(3′)–C(4′)–C(41′)	−69.4(16)	C(73′)–C(74′)–C(79′)–C(78′)	177.9(16)
C(6)–C(7)–C(8)–S(1)	58.0(14)	C(32′)–C(3′)–C(4′)–N(5′)	−66.3(16)	C(75′)–C(74′)–C(79′)–C(78′)	−5.6(25)
N(71)–C(7)–C(8)–S(1)	180.0(9)	C(32′)–C(3′)–C(4′)–C(41′)	55.2(16)	C(74′)–C(75′)–C(76′)–C(77′)	−3.5(31)
C(6)–C(7)–N(71)–C(72)	−157.5(13)	C(3′)–C(4′)–N(5′)–C(6′)	−104.6(15)	C(75′)–C(76′)–C(77′)–C(78′)	3.2(34)
C(8)–C(7)–N(71)–C(72)	84.7(16)	C(41′)–C(4′)–N(5′)–C(6′)	134.3(14)	C(76′)–C(77′)–C(78′)–C(79′)	−4.3(34)
C(4)–C(41)–O(42)–C(43)	−173.0(12)	C(3′)–C(4′)–C(41′)–O(41′)	−92.3(19)	C(77′)–C(78′)–C(79′)–C(74′)	5.4(29)
O(41)–C(41)–O(42)–C(43)	8.3(23)	C(3′)–C(4′)–C(41′)–O(42′)	83.0(16)		

Table 5. Proton chemical shifts and coupling constants for compound (**2a**) in different solvents[a]

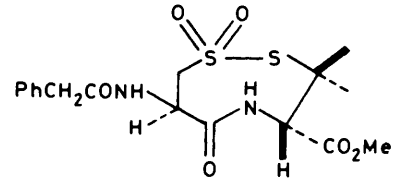
Proton	$CDCl_3$ (p.p.m.)	$(CD_3)_2SO$ (p.p.m.)	Δδ (p.p.m.)[b]	Coupling constants (in Hz in $CDCl_3$)[c]
3-*pro S* CH_3	1.44	1.44	0.00	s
3-*pro R* CH_3	1.48	1.48	0.00	s
8-*pro R* H	2.84	2.90	0.06	14.0, 11.2
8-*pro S* H	3.35	3.14	−0.21	14.0, 5.2
Phenylacetyl CH_2	3.57	3.31	−0.26	s
OCH_3	3.74	3.69	−0.05	s
7-H	4.56	4.68	0.12	11.2, 7.0, 5.2
4-H	4.89	4.76	−0.13	11.1
Cysteinyl NH	6.39	8.40	2.01	7.0
Penicillaminyl NH	6.64	8.81	2.17	11.1

[a] 8 mM Solutions at 25 °C; [b] A negative sign denotes a shift to lower frequency in $(CD_3)_2SO$. [c] s Denotes a singlet.

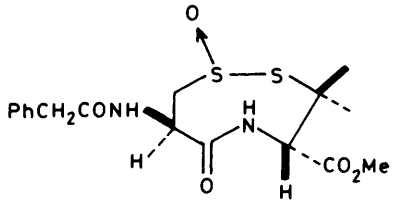
Table 6. Temperature coefficients (p.p.m./°C) for NH resonances of compound (**2a**) in $CDCl_3$ and $(CD_3)_2SO$[a]

Proton	Temperature coefficient (× 10^3) $CDCl_3$[b]	Temperature coefficient (× 10^3) $(CD_3)_2SO$[b]
Cysteinyl NH	−7.5	−6.2
Penicillaminyl NH	−0.1	−3.8

[a] A negative sign indicates a shift to lower frequency with increasing temperature. [b] Calculated for the range −40° to 50 °C in $CDCl_3$ and 20 to 80 °C in $(CD_3)_2SO$



(**6**)



(**7**)

Table 7. Proton chemical shifts and coupling constants for compound (**6**) in $CDCl_3$[a]

Proton	δ (p.p.m.)	Coupling constants (Hz)
3-*pro S* CH_3	1.57	s
3-*pro R* CH_3	1.70	s
8-*pro R* H	3.40	13.9, 11.1
8-*pro S* H	4.15	13.9, 5.2
Phenylacetyl CH_2	3.57	s
OCH_3	3.78	s
7-H	5.11	11.1, 7.2, 5.2
4-H	4.90	11.3
Cysteinyl NH	6.36	7.2
Penicillaminyl NH	7.2	11.3

averaged population of rotamers around the C(7)–N bond, a view reinforced by the observation of a singlet resonance for the side-chain methylene protons. The vicinal coupling constants of the C-8 protons also allow comparison between the solid state and solution conformations. Values calculated using the Karplus-type equations described by Feeney,[17] $J_{7,8pro\ R}$ = 11.9 Hz and $J_{7,8pro\ S}$ = 4.2 Hz, are in reasonable agreement with the observed values of 11.2 and 5.2 Hz respectively, suggesting that the cysteinyl S–C_β–C_α–N angle (*ca.* 180°) is not significantly different in the solution and solid state conformations. However, this data does not preclude an alternative *trans* lactam conformation shown in Figure 3 (conformation II) in which the disulphide bridge adopts a M-helical conformation with a cysteinyl S–C_β–C_α–N torsion angle of *ca.* 110°. Values for $J_{7,8pro\ R}$ and $J_{7,8pro\ S}$ of 3.3 and 11.3 Hz respectively are predicted for this structure by the Feeney equation.

(2) *Nuclear Overhauser Effects.*—The relative proximities of the protons in (**2a**) were determined from n.O.e. difference spectra. The data summarised in Figure 3 can be interpreted in terms of a single disulphide conformation since the barrier to interconversion of P- and M-helical forms is expected to lie in the range 50—90 kJ mol^{-1}.[18,19] If both conformations shown in Figure 3 were in equilibrium we would expect interconversion to be slow on the n.m.r. time scale at 25 °C. Since no such dynamic behaviour is evident from the coupling constant or chemical-shift data only one of the conformations is possible. We show here that the P-helical form (conformation I in Figure 3) is the one adopted in $CDCl_3$ solution.

The penicillaminyl NH shows a significant n.O.e. to 7-H and a small n.O.e. to one of the 3-methyl groups, but no effect to either of the C-8 protons. In the M-helical conformer (II, Figure 3) we would predict an n.O.e. between the lactam NH and the *pro S* proton at C-8. Similarly, in this form a significant n.O.e. between the 8-*pro R* proton, which in this case would have the larger vicinal coupling, and 7-H would be predicted. In fact, 7-H shows an n.O.e. to the C-8 proton with the smaller vicinal coupling indicating a *gauche* relationship between 7-H and the C-8 *pro S* proton. The lack of a measurable n.O.e. between the lactam NH and 4-H is consistent with the *trans* lactam structure. The small n.O.e. between 4-H and the lower frequency methyl group protons is also significant when considered in conjunction with that between the higher frequency methyl group hydrogens and the lactam NH. These suggest a *gauche* relationship between 4-H and the 3-*pro S* methyl group which is only tenable in the P-helical disulphide conformation.

Confirmatory evidence on the solution conformation of (**2a**) was afforded by examination of the solution ^1H n.m.r. spectra of the related sulphone (**6**) which was prepared, together with the corresponding sulphoxide (**7**), by peracid oxidation of (**2a**). In the ^1H n.m.r. spectrum of (**6**) in $CDCl_3$ (Table 7) the resonances associated with the cysteinyl 8-*pro R* and 8-*pro S* protons are shifted to higher frequency as a result of the inductive effect of the adjacent SO_2 group. In addition, the chemical shifts of 7-H, the penicillaminyl NH, and the 3-*pro R* methyl group are observed at higher frequency consistent with deshielding of these protons by a *pseudo* axial (1-*pro S*) oxygen substituent at S-1. The absence of intramolecular H-bonding between this oxygen and the penicillamine NH proton is evident from the appreciable solvent shift for this proton in $(CD_3)_2SO$ (Δδ ~ 2.1 p.p.m., data not shown) comparable with that observed in the

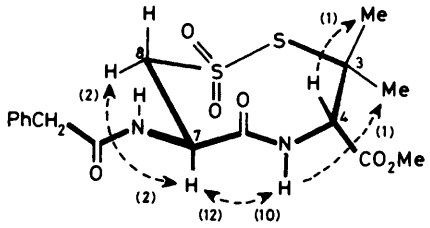


Figure 4. Solution conformation of compound (**6**) showing the principal n.O.e.s observed in the $CDCl_3$ spectrum. Figures in parentheses are the % enhancement values measured in the difference n.O.e. experiment

spectra of (**2a**). These features are consistent only with a rigid P-helical conformation for (**6**) in solution (Figure 4). The similarities in both coupling constant data (see Tables 1 and 3) and the n.O.e.s observed (Figures 3 and 4) for (**2a**) and (**6**) indicate very similar solution conformations for the two molecules.

It seems reasonable to conclude that the solution and solid state conformations of the ring system of (**2a**) are close to identical, being characterised by *trans* lactam and P-helical disulphide functions. It is perhaps noteworthy that in the majority of biological compounds studied to date the P-helical rather than the M-helical disulphide conformation predominates.[7,20]

Experimental

N.m.r. spectra were measured on Bruker WB 360 and WM 200 spectrometers using tetramethylsilane as a standard. I.r. spectra were recorded on a Perkin-Elmer PE 298 spectrophotometer and optical rotations on a Mettler 141 polarimeter. T.l.c. was carried out on 0.25 mm layers of Merck GF_{254} silica. M.p.s, recorded on a Kofler hot-stage apparatus, are uncorrected. Organic solvents were purified and dried by established procedures and organic extracts typically dried over anhydrous $MgSO_4$. Bis-D-penicillamine methyl ester (**4**) was prepared from D-penicillamine and had m.p. 178—180 °C and $[\alpha]_D^{24}$ −10.39° (*c* 2, H_2O) (lit.,[21] m.p. 180—182°; $[\alpha]_D$ −7.5°). *N,N'*-Bisphenylacetyl-L-cysteine (**3**) was prepared as described by Foldi[22] and had m.p. 171 °C and $[\alpha]_D^{24}$ −118° (*c* 1 EtOH) (lit.,[22] m.p. 171°; $[\alpha]_D^{20}$ −120°).

L-*Phenylacetylamino*-4-D-*methoxycarbonyl*-3,3-*dimethyl*-6-*oxoperhydro*-1,2,5-*dithiazocine* (**2a**).—To a solution of the acid (**3**) (2.38 g, 5 mmol) and D-penicillamine methyl ester (**4**) (1.63 g, 20 mmol) in tetrahydrofuran (140 cm^3), triethylamine (1.5 cm^3) and a solution of EEDQ (2.6 g, 10.5 mmol) in tetrahydrofuran (40 cm^3) were added sequentially and the mixture stirred at room temperature for 25 h. The solution was evaporated under reduced pressure, the residue dissolved in EtOAc (50 cm^3), and the resulting solution washed sequentially with aqueous HCl (2M; 20 cm^3 × 2), saturated aqueous $NaHCO_3$ (20 cm^3 × 2), and water (20 cm^3 × 2). The organic extract was concentrated and chromatographed on 2 × 200 × 200 mm silica plates eluted with benzene–EtOAc (2:1) to afford (**5**) which was crystallised from $CHCl_3$–hexane (1.91 g), m.p. 208 °C.

The polymeric dipeptide (**5**) (1.0 g, 1.28 mmol) was dissolved in MeOH–aqueous 5M-HCl (120 cm^3; 5:1) at 0 °C and Zn dust (10 g) added to the cooled stirred solution over a period of 10 min. After a further 30 min, the solution was filtered, concentrated under reduced pressure to 30 cm^3, and poured into cold 1M aqueous NaOH. The basic solution was washed with $CHCl_3$ (50 cm^3) and then taken to pH 1 with concentrated HCl; the acidic solution was then extracted with $CHCl_3$ (100 cm^3 × 3). Evaporation of the $CHCl_3$ afforded the free dithiol as a colourless oil (0.97 g). The dithiol was dissolved in MeOH (70 cm^3), iodine (0.64 g) was added, and the solution refluxed for 2 h; it was then concentrated under reduced pressure to 20 cm^3 and EtOAc (50 cm^3) added. The resultant solution was washed sequentially with saturated aqueous $Na_2S_2O_3$ (50 cm^3 × 2), aqueous HCl (1M; 50 cm^3), aqueous NaOH (1M; 50 cm^3), and water (50 cm^3). Evaporation of the EtOAc layer and crystallisation of the residue from $CHCl_3$ afforded the cyclic disulphide (**2a**) (0.60 g, 1.57 mmol), m.p. 210 °C (transition), remelting at 218—220 °C (Found: C, 53.35; H, 5.8; N, 7.3; S, 16.6. $C_{17}H_{22}N_2O_4S_2$ requires C, 53.40; H, 5.80; N, 7.33; S, 16.73%); $\nu_{max.}$(KBr) 3 290, 1 750, 1 694, and 1 645 cm^{-1}; ^1H n.m.r. see text; *m/z* (e.i.) 382 (M^+, exact mass 382.1021. $C_{17}H_{22}N_2O_4S_2$ requires *M*, 382.1022).

L-*Phenylacetylamine*-4-D-*methoxycarbonyl*-3,3-*dimethyl*-1,1,6-*trioxoperhydro*-1,2,5-*dithiazocine* (**6**).—To a solution of compound (**2a**) (2.54 mg, 0.66 mmol) in $CHCl_3$ (25 cm^3) at 0 °C a solution of 3-chloroperbenzoic acid (160 mg, 1.16 mmol) was added and the solution allowed to come to room temperature over 18 h. The mixture was extracted with saturated aqueous $FeSO_4$ (50 cm^3), saturated aqueous $NaHCO_3$ (50 cm^3), and water (50 ml) and then evaporated. The residue was separated by t.l.c. 1 × 200 × 200 mm silica plates to afford the polar *sulphoxide* (**7**) (100 mg) as prisms from benzene–$CHCl_3$, m.p. 162—167 °C; and the *sulphone* (**6**) (98 mg) as prisms from $CHCl_3$–hexane, m.p. 191—192 °C (Found: C, 47.2; H, 5.15; N, 6.4; S, 14.8. $C_{17}H_{22}N_2O_6S_2 \cdot H_2O$ requires C, 47.22; H, 5.55; N, 6.48; S, 14.81); $\nu_{max.}$(KBr) 3 280, 1 755, 1 700, and 1 655 cm^{-1}; ^1H n.m.r. see text; *m/z* (e.i.) 414 (M^+), 382, 349, 316, 277, and 91.

Crystal Structure of Compound (**2a**).—*Crystal data.* $C_{17}H_{22}N_2O_4S_2$, *M* = 382.5, space group *P*1, *a* = 9.240(4), *b* = 9.642(4), *c* = 11.317(4)Å, α = 94.97(5), β = 98.85(5), γ = 110.37(5)°, *V* = 923.2Å^3, *Z* = 2, D_x = 1.35 g cm^{-3}, D_c = 1.38 g cm^{-3}, Mo-K_α radiation, λ = 0.710 69, μ = 1.99 cm^{-1}, *F*(000) = 404, *T* = 293 K.

Data collection and processing: structure analysis and refinement. 3 246 Independent data were collected on an Enraf-Nonius CAD-4 diffractometer to a maximum sinθ/λ of 0.059 Å^{-1}. The structure was solved and refined using the 1 256 data with $I/\sigma(I) \geqslant 3.0$. The data give a nearly centric intensity distribution, and the Patterson function indicated a centric arrangement of the four S atoms in the unit cell. The enantiomorph was fixed and the structure expanded to all but two non-hydrogen atoms by two cycles of the DIRDIF procedure.[23] After correction of the absolute configuration, the remaining carbon atoms and several hydrogen atoms were found in a difference Fourier synthesis. Hydrogen atoms were included in the refinement in idealised positions with thermal parameters riding on those of the carbon atoms to which they were bonded. Hydrogen atoms bonded to nitrogen were refined positionally with temperature factors fixed at *U* = 0.005.[2] In the final cycles, S, O, and N atoms were refined anisotropically. In the final refinement cycle, a weighting scheme $w = 1.38/[\sigma^2(F) + 0.000\,36F^2]$ was used to refine 290 parameters. On this cycle, the maximum shift/error for a parameter was 0.19, the final agreement factors were *R* = 0.051, R_w = 0.053, and a final difference Fourier synthesis gave maximum peak and trough of 0.34 and −0.24 e Å^{-3} respectively. Fractional co-ordinates for hydrogen atoms and thermal parameters are available from the Cambridge Crystallographic Data Centre on request.*

* See 'Instructions for Authors 1988,' *J. Chem. Soc., Perkin Trans. 1*, 1988, Issue 1.

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