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98. A Chiral Cystine Disulfide Group without Inherent Optical Activity in the Long-Wavelength Region

(^{1}H- and ^{13}C-NMR., UV., CD., and ORD. Studies with *cyclo*-L-Cystine)

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Zusammenfassung. Konformation und Konfiguration von *cyclo*-L-Cystin wurden mit spektroskopischen Methoden untersucht. Nach ^{1}H- und ^{13}C-NMR. besitzt die Molekel einen wannenförmigen Diketopiperazin-Ring (C_αH–NH-Diederwinkel $\simeq 40°$) und eine *chirale* Ausbildung der Disulfidbrücke. In Molekelmodellen mit diesen Spezifikationen beträgt der C-S-S-C-Diederwinkel $\varphi \simeq 90°$. Im CD. war aber kein langwelliger *Cotton*-Effekt zu beobachten, welcher auf eine inhärente optische Aktivität des asymmetrischen Disulfid-Chromophors hinwiese. Dieses Verhalten stützt die Theorie von *Linderberg & Michl* [3b], wonach bei Disulfiden mit $\varphi = \pm 90°$, infolge der Entartung der beiden Elektronenübergänge niederer Energie und der irreduziblen Darstellung *A* bzw. *B*, die sonst getrennten Absorptionsbanden bei 250 nm zusammenfallen und ihre entgegengesetzten Rotationsstärken sich aufheben.

Den starken, negativen *Cotton*-Effekt bei 228 nm (CD., in Äthanol) ordnen wir dem n,π^*-Übergang der Peptid-Carbonylgruppe zu. Vorzeichen und Rotationsstärke ($R = -45{,}4 \times 10^{-40}$ erg · cm^3) dieser Bande deuten wir als starke Indizien für das Vorliegen des *cyclo*-L-Cystins als *P*-helikales Diastereomeres.

1. Introduction and Conclusions. – The disulfide chromophore is known to have two UV. absorption regions: one at about 180–200, and the other at about 220–300 nm. The long-wavelength absorption is dependent on the disulfide dihedral angle φ [2], exhibiting either one band at roughly 245–250 nm ($\varphi \simeq \pm 90°$) or two bands positioned symmetrically at each side of this value. These phenomena can be explained by assuming one or more angle-independent transitions (σ, σ^*?) to be

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responsible for the short-wavelength, and two angle-dependent transitions (ψ_+, σ^* and ψ_-, σ^*) for the long-wavelength bands [3a, b]. The two transitions, ψ_+, σ^* and ψ_-, σ^*, become degenerate ($\psi_\pm$, σ^*) for $\varphi = \pm 90°$ ($\tilde{\nu} \simeq 40{,}000\ cm^{-1}$), but have different energies for $\varphi \neq \pm 90°$, depending on $\cos\varphi$ (Fig. 1). Hence, the band separation increases as φ deviates from $\pm 90°$ and approaches either 0° or 180°. For cisoid disulfides ($|0°| < |\varphi| < |90°|$) the $\lambda_{max} > 250$ nm band is supposed to correspond to ψ_-, σ^*, for transoid ($|90°| < |\varphi| < |180°|$) to ψ_+, σ^* (*vice versa* with the $\lambda_{max} < 250$ nm long-wavelength band).

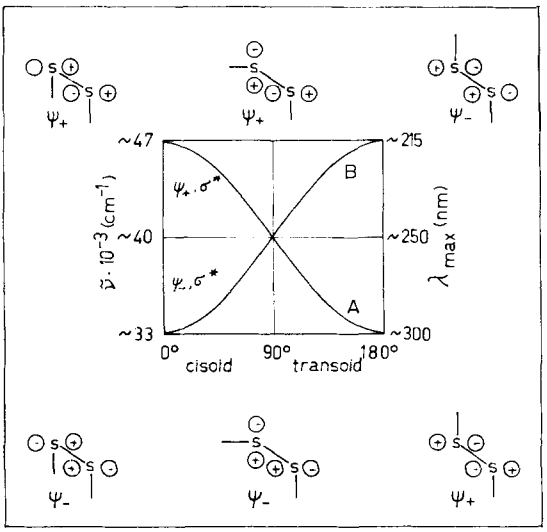


Fig. 1. *Approximate excitation energies, $\tilde{\nu}$, and irreducible symmetry species of electronic transitions from ψ_+ (bonding at $\varphi = 0°$, antibonding at $\varphi = 180°$) and ψ_- (antibonding at $\varphi = 0°$, bonding at $\varphi = 180°$) disulfide orbitals to a virtual σ^* orbital*

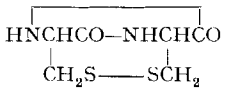
Dependence of UV. absorption maxima, ν and λ, on φ_{SS}. Schematic representations of the orbital phase relations at $\varphi_{SS} = 0°$, 90° (degeneracy), and 180°. The following relations hold between transition symmetry (A, B), rotational strength (R), and helical chirality (M, P) for the individual absorption bands: $A: -R \equiv P, +R \equiv M$; $B: +R \equiv P, -R \equiv M$ [3b, 6]. At $\varphi_{SS} = 90°$, $-R(A)$ and $+R(B)$, or $+R(A)$ and $-R(B)$ of the P or M helical forms, respectively, are superposed at about 250 nm and cancel out [$R(A) + R(B) \simeq 0$]

Two regions of inherent optical activity corresponding to those of UV. absorption are also known. The angle dependence of the *Cotton* effects is poorly understood in the 200 nm [4], but quite well so in the 220–300 nm range [3b]. The ψ_+, σ^* and ψ_-, σ^* transitions can be shown to have different symmetries with respect to the disulfide C_2 axis (irreducible species A and B, respectively). This situation, which is somewhat similar to that in twisted ethylenes [5], produces a *Cotton* effect couplet with the rotational strength, R, of an individual CD. band negative for right-handed (P) and positive for left-handed (M) helical disulfide chirality in the case of transition symmetry species A (ψ_+, σ^*), and *vice versa* for B (ψ_-, σ^*); cf. [6]. Thus, in *cisoid* disulfides, P-helicity is indicated by $(+)R$, in transoid by $(-)R$ of the longest-wavelength *Cotton* effect. Model compounds have been provided by *Carmack* & *Neubert* and others [7]

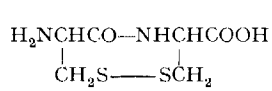
and by *Ludescher & Schwyzer* [8] for the two angle ranges, respectively, providing strong support for this "quadrant rule" (for an illustration cf. [8]) and the general theory of disulfide chiroptics [3b][2]).

According to this theory, chiral disulfides with $\varphi = \pm 90°$ should exhibit UV. absorption at $\lambda_{max} \simeq 250$ nm (ψ_+, σ^*) and be devoid of inherent optical activity in the long-wavelength region. Theoretically, the two *Cotton* effects arising from ψ_+, σ^* and ψ_-, σ^* should, for a given compound with $\varphi \neq 90$, 180, or 0°, have opposite sign and – lacking other major perturbations – equal magnitude. In compounds with $\varphi = \pm 90°$, degeneracy (to $\psi_\pm$, σ^*) would superpose the two *Cotton* effects and abolish optical activity despite chirality. We present here, for the first time, evidence that this intuitively unexpected prediction is correct, thus further substantiating the *Bergson-Lindenberg-Michl* disulfide theory.

Cyclo-L-cystine **1** has recently been prepared from the methyl ester of L-cysteinyl-L-cysteine disulfide **2** [1]:



1



2

The ease of **2** → **1** cyclisation agrees with the postulated presence of the *cis* peptide bond in **2** (energy calculations [10]). Molecular models of **1** and **2** can be built with

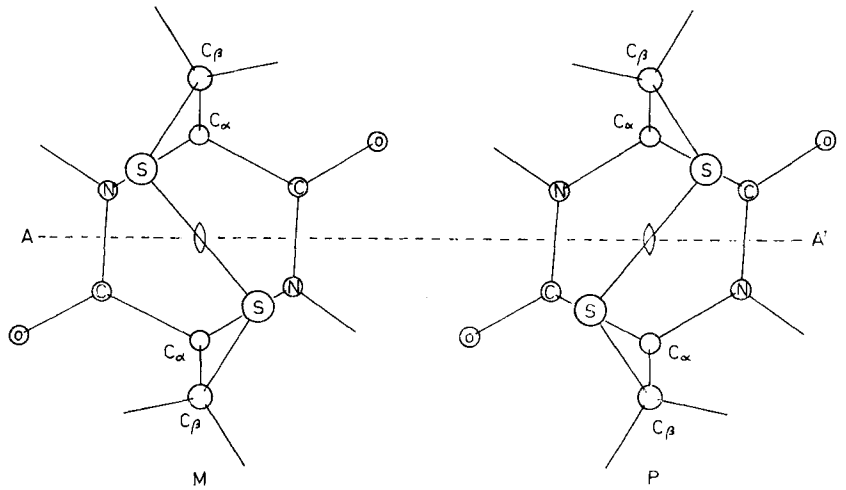


Fig. 2. *Model representation of* M- *and* P-*helical cyclo*-L-*cystine* **1** *viewed along the* C_2 *axis* (*O*)
Half-cystine residue No. 1 appears above, No. 2 below the plane containing AA' and the C_2 axis. The atoms are designated accordingly, e.g. O^1, O^2. Some interatomic distances (values of a corresponding planar diketopiperazine in parentheses): C^1_β–O^1 3.1 (2.8); C^2_β–O^1 4.4 (4.5); *M*: S^1–O^1 4.3, S^2–O^1 4.0; *P*: S^1–O^1 3.5, S^2–O^1 4.8 Å

[2]) Without knowing the dihedral angle range (cisoid or transoid), it is impossible to determine the chirality of a given disulfide group from CD. data alone, *e.g.* the assignment of *P*-helicity to oxytocin and related hormones [9] must, although it might be correct, remain equivocal.

equal ease by incorporation of either an *M*- or a *P*-helical disulfide bridge with $\varphi = \pm 90°$ (Fig. 2).

The CD. of **1** revealed no *Cotton* effect in the 240–300 nm region, which could have been ascribed to inherent optical activity of the disulfide chromophore. This behaviour could be due to the presence either of an equimolar mixture of the two diastereomers of **1** with opposite chirality of the disulfide bridge (trivial), or of only one (or an excess of one) of the helical forms (according to theory, but hitherto unobserved).

^{1}H-NMR. studies showed that the diketopiperazine ring of **1** is in a boat conformation as in the model and Fig. 2 (NH–C_αH coupling [11]) and that the disulfide bridge assumes only one of the two possible chiral configurations ($C_\beta H_2$–C_αH chemical shifts and coupling constants). The ^{13}C–NMR. spectra agree with this interpretation and prove the exact C_2 symmetry of the molecule. The position of the long-wavelength UV. absorption band of **1** at 247 nm supports the assignment of $\varphi = \pm 90°$ for the disulfide bond.

Thus, *cyclo*-L-cystine appears to be the first example corroborating the 90° statement of *Linderberg & Michl's* "quadrant rule" [3b].

It is more difficult to decide on the screw sense of the disulfide helix in **1**: NMR. provides only unreliable clues, but CD. more definitely indicates the *P*-helical arrangement (which would also appear to be favoured from thermodynamical considerations). We ascribe the *Cotton* effect at 228 nm to the peptide n, π^* transitions. Sign and magnitude of R (-45.4×10^{-40} erg·cm^3) seem to imply the proximity and dominant influence of the highly polarisable hemicystine side chain (–CH_2–S–) in a *positive* octant. This is the situation in the *P*-helical model (Fig. 2) in which the other hemicystine group in the negative octant is more remote. In the *M*-helical molecule, however, the two sulfur atoms are at almost equal distances from the carbonyl oxygens. Although we believe that in the latter case R should be much less negative (or even slightly positive), we cannot be certain, because the magnitude of the rigidity influence introduced by the bridge is only roughly known. Complete clarification will probably require further synthetic models.

2. Materials and Methods. – **1** was the carefully recrystallised product described in [1]. ^{1}H-NMR. spectra were determined in hexadeuterio-dimethylsulfoxide ($DMSO_{d6}$) at 100 and at 220 MHz (*Varian* XL-100 and HR-220). The peptide concentration was 30 mg/ml (0.147 M). The temperature was measured with an ethylene glycol probe. Amide protons were exchanged against deuterons by adding a few drops of deuterio-trifluoroacetic acid (TFA_d). Hexamethyl-disiloxane served as an internal reference. The *ABC*-spectra were computer-simulated with the NMR.8P programme. For determination of the ^{13}C-NMR. spectra, 37.5 mg of **1** were dissolved in 0.4 ml of $DMSO_{d6}$. The ^{1}H-NMR. spectrum of this extensively purified material was identical with that of all previously examined samples, i. e. it contained approximately the same amount of the 'impurity' observed before (cf. 3.1 and 4.1). ^{13}C-NMR. spectra at 25.14 MHz were obtained on the *Varian* XL-100 spectrometer using the *Fourier* Transform technique. A sample tube of 5 mm outer diameter was used; sample temperature $\simeq 28°$. Chemical shifts are relative to internal tetramethylsilane, the centre of the multiplet of $DMSO_{d6}$ being at -39.8 ppm. The system was locked on the ^{2}H signal of the solvent. Ultraviolet (UV.), optical rotatory dispersion (ORD.), and circular dichroic (CD.) spectra were determined in 96 per cent ethanol on a *Beckman* DK 2A, a *JASCO* ORD/UV-5, and a *Jouan-Roussel* Dichrograph instrument, respectively.

3. Results. – 3.1. *The ^{1}H-NMR.* ABC *and* ABCX *spin systems.* **1** contains 8 protons in two equivalent *ABCX* spin system (Figs. 3, 4). The two amide protons give rise to a broad singlet at 8.03 (*X*), the two C_α protons to a symmetrical quartet centered at 4.12 (*C*), and the 4 C_β protons to an asymmetrical multiplet with 6 resolved lines between 2.9 and 3.6 ppm (*AB*). The weak signals at

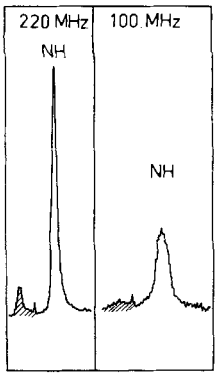


Fig. 3. *NMR.-spectra of* **1** *in the amide proton region*
Main resonance at 8.03, 'impurity' (shaded) at 8.31 ppm

3.11, 4.19, and 8.31 ppm are well separated at 220 MHz and were assigned to an impurity which could not be removed by recrystallisation and did not impair the elemental analysis results [1]. The resonance at 3.36 ppm is probably due to (bound ?) HOD.

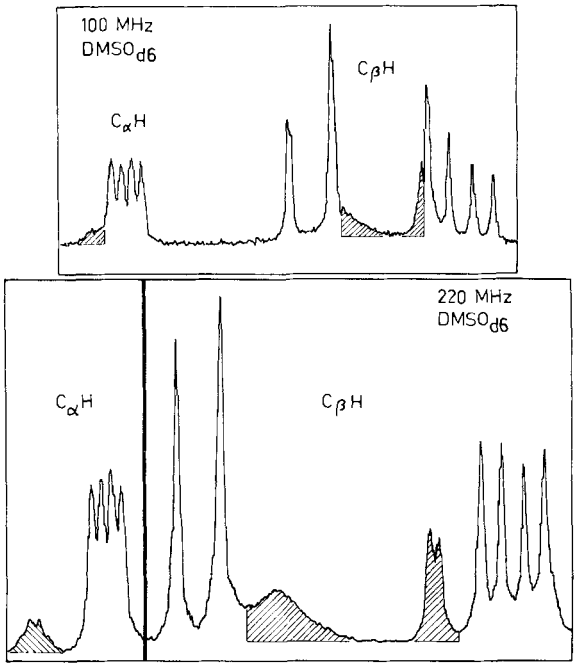


Fig. 4. ^1H*-NMR. spectra of* **1** *in the* $C_\alpha H$ *and* $C_\beta H_2$ *region*
Main resonances: $C_\alpha H$ (quartet) centered at 4.12 ppm; $C_\beta H_2$ between 2.9–3.6 ppm. 'Impurity' signals (shaded) at 3.11, 3.36, and 4.19 ppm

Addition of TFA_d changes the *ABCX* to an *ABC* spectrum (Fig. 5a). The *C* part is now a doublet with a line separation of 6.8–7.0 Hz. The *AB* part can be resolved into two overlapping, symmetrical quartets (lines 1, 3, 5, 7 and 2, 4, 6, 8) if it is assumed that two resonances are contained in each of the two downfield signals (almost complete coincidence of lines 7 and 8, and 5 and 6, respectively). The chemical shifts, δ, and the coupling constants, J, were calculated according to

Pople et al. [12]: $\delta_A = 304.2$, $\delta_B = 353.8$, $\delta_C = 414.0$ Hz (at 100 MHz); $J_{AC} = \pm 7.0$, $J_{BC} = \pm 0.0$, $J_{AB} = \pm 14.3$ Hz.

The spectrum of Fig. 5b was obtained from these values by computer simulation. The agreement with Fig. 5a is excellent and justifies the assignment of the impurity bands.

Fig. 6 shows the *Newman* projections of C_β on C_α for the *M*- and *P*-helical configurations of the disulfide bond in **1**, taking into account that the dihedral angle between C_α–H_C and either C_β–H_A

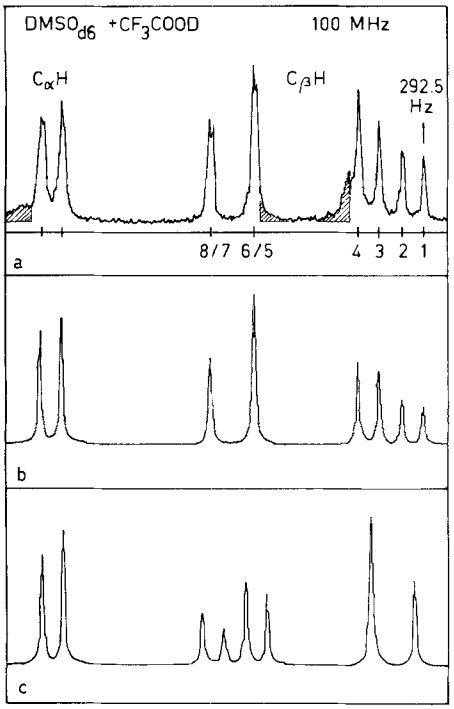


Fig. 5. *^{1}H-NMR. spectra of* **1** *(in $DMSO_{d6}$ + TFA_d) in the $C_\alpha H$ and $C_\beta H_2$ region*

a) Observed at 100 MHz (shaded 'impurity' resonances). b) Simulated with $J_{AB} = 14.3$, $J_{AC} = \pm 7.0$, $J_{BC} = \pm 0.0$, $\delta_A = 304.2$, $\delta_B = 353.8$, $\delta_C = 414.0$ Hz. c) Simulated with the same chemical shifts, but with exchanged vicinal coupling constants: $J_{AC} = \pm 0.0$, $J_{BC} = \pm 7.0$, $J_{AB} = \bullet 14.3$ Hz

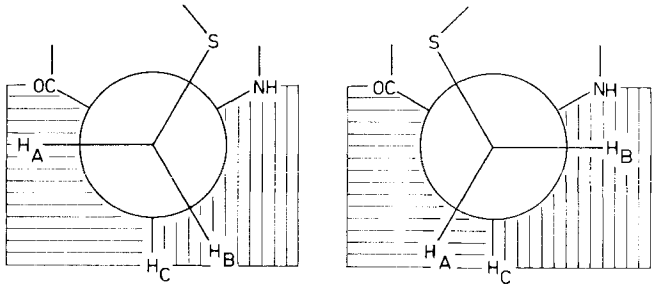


Fig. 6. *Newman-projection of C_β on C_α in* **1** *with* M- *and* P-*helical configurations of the disulfide bond*
Approximation for interpreting the NMR. data: Chemical shifts of C_β-H in horizontally differ from those in vertically shaded sectors, but are practically invariant for the two positions within *one* area (through-bond anisotropic effects of N and CO). *Assumption* for the calculation of chemical shifts and coupling constants: H_A is influenced by CO, H_B by NH, producing $\delta_A = 304.2$ and $\delta_B = 353.8$ Hz. $J_{AC} \simeq \pm 7.0$, $J_{BC} \simeq \pm 0.0$ Hz in *P* and vice versa in *M*

or C_β–H_B must be 90° ($J \simeq 0$ Hz). *The assignment of subscripts A and B is tentative*: on the assumptions made in Fig. 6, configuration *P* would correspond to the simulated spectrum 5b (an exchange of the two subscripts would make 5b correspond to *M*).

In order to simulate a spectrum for the *other* configuration (in our case *M*), it was *assumed* that (as a first approximation) the rotation of 60° required to change *P* to *M* would leave the protons marked H_A and H_B under the influence of the CO and NH groups, respectively, *without appreciably changing their chemical shifts*. The only necessary alteration would then be the exchange of the coupling constant values of J_{AC} and J_{BC} (cf. legends to Figs. 5, 6). A calculation based on this change produced the spectrum in Fig. 5c which is clearly different from that observed, 5a.

3.2. *The ^1H-NMR. CX spin system.* The vicinal coupling constant between the amide and C_α protons was determined from a comparison of Fig. 4 with Fig. 5: $J_{NC} = 3.2$ Hz. A *Karplus*-type treatment [11] resulted in a value of $\simeq 40°$ for the dihedral angle in both NH–C_αH pairs.

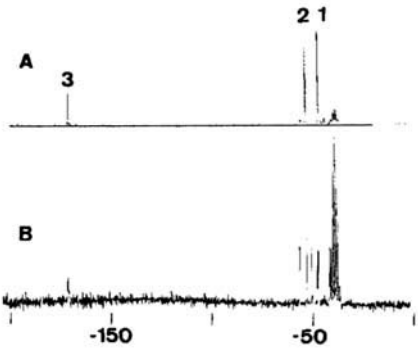


Fig. 7. *^{13}C-NMR. spectra of* **1** *at 25.15 MHz*

37.5 mg in 0.4 ml $DMSO_{d_6}$. 124,000 pulses were accumulated: pulse width 75 μsec, acquisition time 0.4 sec, pulse delay 0.6 sec. A: with, B: without proton noise decoupling by irradiation at 100 MHz. The septet at -39.8 ppm is from $DMSO_{d_6}$; resonances 1, 2, and 3 correspond to C_β, C_α, and carbonyl-C of **1**, respectively

3.3. *^{13}C-NMR. spectra.* Fig. 7 shows the ^{13}C-NMR. spectra of **1** with and without noise decoupling of protons. The resonance assignments follow from the chemical shifts $\delta_1 = -48.0$ (C_β), $\delta_2 = -54.1$ (C_α) and $\delta_3 = -170.9$ (CO) ppm, and from the multiplet structure of the resonances.

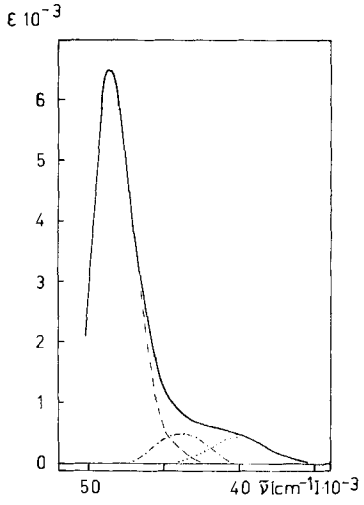


Fig. 8. *UV. spectrum of* **1** *in ethanol*

Tentative resolution: disulfide $\psi_{\perp}$, σ^* (·····), peptide n,π^* (–·–·–·), peptide π,π^* (– – –)

The coupling constants between C(1) (C_β) and the methylene protons are $J_{C(1)H} = 148$ and $J_{C(1)H'} = 139$ Hz; $J_{C(2)H}$ is 145 Hz (C_α). Long-range proton to ^{13}C spin-spin coupling is clearly manifested in the spectra, which indicates that the long range coupling constants are of the order 5–15 Hz for the various possible interactions.

The two hemicystyl residues are equivalent in the ^{13}C-NMR. spectrum. From the resonance line widths the following upper limits for the chemical shift differences were obtained: $|\delta_1 - \delta_{1'}| > 0.3$, $|\delta_2 - \delta_{2'}| < 0.4$, $|\delta_3 - \delta_{3'}| < 0.2$ ppm.

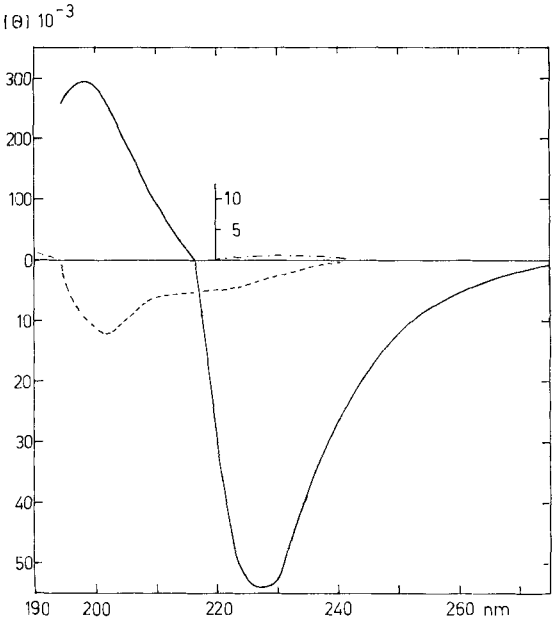


Fig. 9. *CD. spectrum of* **1** *in ethanol* (———)
For comparison: *cyclo* (-Ala-Gly-) [24] in ethanol (– – – –), and *cyclo* (-Ala-Ala-) [25c] in water (–·–·–·)

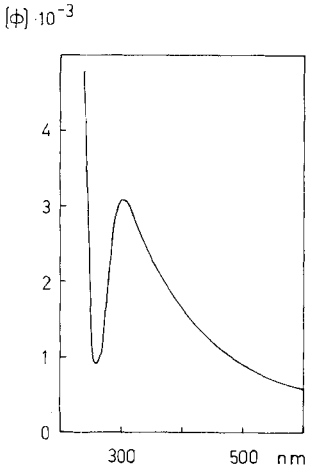


Fig. 10. *ORD. spectrum of* **1** *in ethanol*

The impurities observed in the ^{1}H-NMR. spectrum might be responsible for the weak ^{13}C resonances at -56.8 and -44.8 ppm. No resonance was detected corresponding to an 'impurity' carbonyl carbon atom.

3.4. *UV. spectra* (*Fig. 8*). The following concentrations of **1** were used (moles/l): $1.973 \cdot 10^{-4}$ (200–210 nm), $4.932 \cdot 10^{-4}$ (215–255 nm), $4.932 \cdot 10^{-3}$ (260–300 nm). A peak was observed at 205.3 nm, $\varepsilon = 6477\,l \cdot mole^{-1} \cdot cm^{-1}$, and a shoulder at approximately 250 nm, $\varepsilon = 474$.

3.5. *CD.* (*Fig. 9*). A concentration of $5.027 \cdot 10^{-4}$ and a path-length of 1.0 mm were used to scan the region of 185–300 nm; $5.027 \cdot 10^{-3}$ at 0.1 mm (185–300) and 10 mm (240–300 nm) served as controls. Two molar ellipticity extrema, $[\Theta] = -49{,}800$, $R = -45.4 \cdot 10^{-40}$ (228 nm) and $+296{,}000$ deg · cm^3 · decimole^{-1}, $R = +81 \cdot 10^{-40}$ erg · cm^3 (198 nm), were recorded. No differences due to concentration changes were observed.

3.6. *ORD.* (*Fig. 10*). Concentrations of 0.100 mg/ml (200–240 nm) and 1.00 mg/ml (240–600 nm), and pathlengths of 0.1 dm were used. Extrema were registered at 300, 260, and 200 nm with molar rotations, $[\Phi]$, of $+3105$, $+858$, and $+120{,}000°$, respectively.

4. Discussion. – 4.1. *^{1}H-NMR.* Molecular models of **1**, without any strain (Figs. 2, 11, 12), can be built from commercially available sets (*CPK*, *Dreiding*, etc.). They contain the observed NH–C$_\alpha$H dihedral angle of $\simeq 40°$, and the disulfide bond in either *M*- or *P*-helical configuration with dihedral C$_\beta$–S–S–C$_\beta$ angles of $\varphi \simeq \pm 90°$. One of the two C$_\beta$ protons of each half-cystine residue is placed at a dihedral angle of $\simeq 90°$ to the C$_\alpha$ proton, the other at $\simeq 30°$. This agrees with the vicinal coupling constants of the *ABC* system, $\simeq 0$ and 7 Hz.

The NMR. spectrum calculated from the chemical shifts and coupling constants of the *ABC* spin system (Fig. 5b) agrees very well with the observed spectrum. Assuming that this spectrum corresponds to *one* of the diastereomers of **1**, the spectrum of the *other* (Fig. 5c) was calculated by exchange of the vicinal coupling constants, but leaving the chemical shifts unchanged; this spectrum differs strongly from that observed, 5a. We take this as an indication that **1** exists mainly in one, thermodynamically favoured configuration (*M* or *P*, Fig. 6). If 1 were a mixture of substantial amounts of *M* and *P*, its spectrum should in general be different from both 5b and 5c[3]).

From the ^{1}H–NMR. data alone, we can only speculate on which of the two diastereomers corresponds to reality. We should expect the amide protons in *P* to be more shielded by the sulfur "lone pair" electrons than in *M*. From our knowledge of chemical shifts of amide protons in diketopiperazines [13] we cannot reach a decision. However, the juxtaposition of the NMR. signals, the extreme difficulty of removal, and the excellent analytical data could mean that the impurity present in **1** was a small amount of the other, less favoured diastereomer. If this was the case, the amide protons of the major would appear to be shielded by $\simeq 0.3$ ppm with respect to the minor diastereomer, suggesting that **1** was identical with *P*, the impurity with *M* (Fig. 3). The *P* helical disulfide arrangement could also explain qualitatively the thermodynamic preference of **1** over the minor diastereomer: in *P*, the polarisable disulfide bond is oriented approximately parallel to the local peptide dipoles [14], and perpendicular in *M* (Fig. 2, 11), suggesting a stabilisation of *P*

[3]) If, in the nomenclature of Fig. 6, δ_A (of conformer *M*) $= \delta_B(P)$, $\delta_B(M) = \delta_A(P)$, $J_{AC}(M) = J_{BC}(P)$, and $J_{BC}(M) = J_{AC}(P)$, the simulated spectrum 5b would correspond to either of the two conformers *M* and *P* (Figs. 2, 6) or a mixture thereof. However, in view of the asymmetric properties of the C$_\alpha$-carbon atom, this possibility seems rather improbable.

relative to *M*. The CD. results support this tentative assignment, which the ^{13}C-NMR. does not contradict.

4.2. *^{13}C-NMR.* These data appear to provide further evidence that only one of the two possible intramolecular disulfide bridge arrangements is actually (mainly) present. In *P* and *M* (Fig. 2, 6) the rotation angles about the C_α–C_β bond are different; because of the asymmetry at the α-carbon atom, which is clearly manifested in the magnetic non-equivalence of the two β-methylene protons, the ^{13}C chemical shifts should in principle be different in the two diastereomers.

In previous experiments, *Jung et al.* [15] observed a downfield shift of $\simeq -13$ ppm for the β-carbon resonances when changing from linear cysteinyl to the corresponding linear cystyl compounds, the respective resonance positions being approximately -26 to -27.5, and $-38{,}5$ to -43.0 ppm. The β-carbon resonance of **1** at -48.0 ppm is clearly outside these two regions. A possible explanation is offered by the rather pronounced rigidity of **1**. The β-carbon atom(s) might thus spend most of the time in a conformation which corresponds to an extreme low field position of the ^{13}C resonance. In the more flexible molecules studied by *Jung et al.*, the observed resonance positions correspond to the average of all possible conformations, which may include a much larger range of rotational angles about C_α–C_β than in **1**.

The "impurity resonances" (C_α and C_β *different*, CO *indistinguishable* from those of **1**) do not exclude the possibility of the impurity being identical with the minor diastereomer.

4.3. *UV.* The UV. absorption spectrum of **1** (Fig. 8) can easily be explained in terms of peptide and disulfide chromophores. The closely related compound, L-2,5-dimethyl-diketopiperazine (alanine anhydride) in water shows one *peptide band* at $\lambda_{max} = 188$ nm ($\varepsilon = 16{,}500$) which is supposed to result from a superposition of the strong amide π,π^* ($\lambda_{max} < 200$ nm) and the very weak n,π^* ($\lambda_{max} \simeq 210$–220 nm in hydroxylic solvents, $\varepsilon \simeq 50$–150) transitions [16]. The *disulfide absorptions* of cystine and 2,2'-dithio-diethanol in water exhibit bands at 187 ($\varepsilon \simeq 6000$) and 246 nm ($\varepsilon \simeq 300$) [4]. The *short-wavelength band* of **1** presumably contains contributions of both the peptide and the disulfide chromophors. It is batho- (+17 nm) and hypochromic ($\simeq 40\%$) relative to alanine anhydride. We have not investigated whether this difference is due only to a change of ring conformation or to an influence of the disulfide group also: non-planarity as in **1** would by itself be expected to enhance the mutual strong electric dipole interactions between the peptide π,π^* transitions [17] as compared to the time-average planar molecules of highly flexible [18] alanine anhydride and to produce such effects. The *long-wavelength shoulder* at about 215–290 nm is reasonably explained by superposition of amide n,π^* and disulfide ψ_+, σ^* transitions. A tentative resolution is offered in Fig. 8. The disulfide absorption would be centered at about 247 nm ($\varepsilon \simeq 500$) and the amide n,π^* band at $\simeq 228$ nm (this position was chosen because of the CD. results; $\varepsilon \simeq 500$). The values of ε are *very* uncertain, because of the unknown tailing of the strong 205 nm band. The n,π^* absorption is known to lie between 225–235 nm in the case of simple amides in solvents like cyclohexane and dioxane and to be shifted to higher energies in hydroxylic solvents [19]. The relatively low energy of this transition in **1** in ethanol could possibly be due to the disulfide group acting as a "fixed" molecule of non-hydroxylic, easily polarisable "solvent", resulting in a reduction of hydrogen bonding and, hence, ground

state stabilisation. However, we have no further experiments supporting this hypothesis.

The observation of long-wavelength disulfide absorption at $\lambda_{max} \simeq 247$ nm (and none at lower energy) is, according to *Barltrop et al.* [2], strong evidence for $\varphi_{SS} = \pm 90°$, the value also suggested by the NMR.-compatible models of **1**.

4.4. *CD.* The CD. spectrum of **1** would *a priori* be expected to show *Cotton* effects corresponding to its disulfide and peptide absorptions, and hence be related to that of similar compounds containing these chromophores.

[2,7-Cystine]-gramicidin S is a cyclic decapeptide with a disulfide bridge fixed in the *P*-helical configuration with $\varphi_{SS} \simeq +120°$. Its CD. difference-spectrum against [2,7-bis-(S-acetamidomethyl-cysteine)]-gramicidin S exhibits essentially three *Cotton* effects arising from disulfide inherent optical activity: $R_{271.5\,nm} = -12.3 \cdot 10^{-40}$ (ψ_+, σ^*; A), $R_{230\,nm} = +58.6 \cdot 10^{-40}$ (ψ_-, σ^*; B), and $R_{202\,nm} \simeq -75 \cdot 10^{-40}$ erg·cm^3 (σ, σ^*?) [8]. The disulfide chromophore of N,N'-diacetyl-L-cystine-bismethylamide (DACMA) has, according to a computed resolution of its complex spectrum, two CD. ellipticity minima at $\simeq 199$ ($R = -33.7 \cdot 10^{-40}$) and $\simeq 262$ nm ($R = -1.64 \cdot 10^{-40}$) [4] (in view of the very low rotational strength and strong peptide superposition, an expected positive peak around $\simeq 238$ nm might have been missed).

The short-wavelength peak of **1** is located at virtually the same position as that of DACMA, but differs in sign and magnitude ($R = +81 \cdot 10^{-40}$), Fig. 9. It might be the result of superposed peptide (π, π^*) and disulfide (σ, σ^*?) contributions. The conformational rigidity of the molecule would be expected to enhance the disulfide rotational strength relative to DACMA [19b]. This phenomenon is well documented with the pair [2,7-cystine]-gramicidin S/DACMA. In view of the angle between the planes of the two *cis*-peptide bonds in **1**, a rather strong electric dipole-dipole interaction producing optical activity is to be expected (cf. 4.3 and for a lucid presentation [20]). However, we have not been able to resolve the short wave-length *Cotton* effect into peptide and disulfide contributions, a prerequisite for the study of such mutual interactions.

A peak corresponding to the long-wavelength disulfide *Cotton* effects of DACMA or [2,7-cystine]-gramicidin S is clearly missing in **1**, as shown by a number of careful determinations of CD. spectra with different concentrations. The strong minimum found at 228 nm ($R = -45.9 \cdot 10^{-40}$) seems to correlate with the 220–227 nm (aqueous solutions) bands of DACMA, N,N'-diacetyl-L-cystine, and oxidised glutathione,

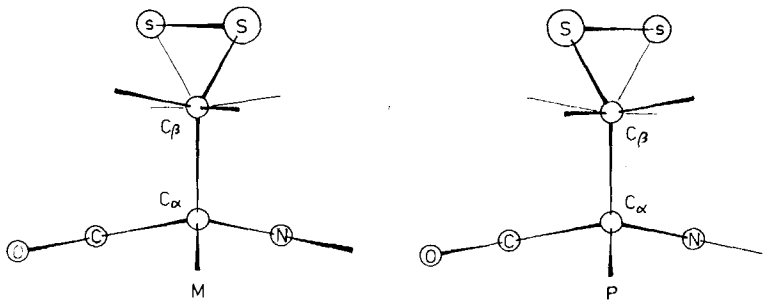


Fig. 11. *Model representation of* **1**, M *and* P, *viewed perpendicularly to the* AA'–C_2 *plane* (Fig. 2)

which were assigned to amide n,π^* transitions [4]. As stated above, the *Linderberg-Michl* theory [3b] offers a reasonable explanation for this lack of long-wavelength disulfide inherent optical activity despite chirality[4]).

On the reasonable assumption that the 228 nm Cotton effect of **1** results primarily from *perturbed peptide* n,π^* *transitions*, we can tentatively draw further conclusions about the molecular geometry. To this end, **1** can be regarded as disulfide derivative of L-alanine anhydride. Any CD. differences would then arise from: a) the accentuated non-planarity and rigidity of the diketopiperazine ring caused by the disulfide bridge, and b) the substitution in the side chains and the disposition in space of the substituent disulfide group. Figs. 2, 11, and 12 are relevant to the following discussion. The orientation of the molecule relative to the coordinate system for the local n,π^* transitions is specified in the legend to Fig. 12.

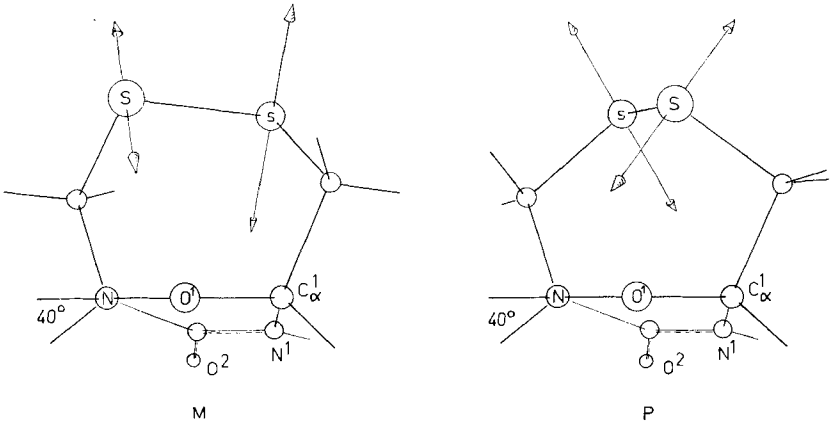


Fig. 12. *Model representation of* **1**, M *and* P, *viewed along the* O^1–C^1 *bond*

The dihedral angle N^2H–C^2H appears at left. The arrows indicate the direction and approximate *van der Waals* dimension of the sulfur 'lone pair' orbitals. In the discussion of n,π^* transitions (text), each carbonyl oxygen is assumed to lie at the origin of an orthogonal coordinate system: in this Fig. the viewer is looking along the z-axis of the O^1 coordinate system, the positive directions of the y and x axes are horizontally to the right and perpendicularly upward, respectively

According to the model represented in Fig. 12, the carbonyl oxygen O(1) will be perturbed by at least 3 types of groups: the peptide, CO(2)–N(1), the methylene, $C(1)_\beta$ and $C(2)_\beta$, and the disulfide groups. Because of the exact C_2 symmetry, the perturbation of carbonyl oxygen O(2) should be identical with that of O(1), because the perturbing groups are indistinguishable, except for their indices. Methylene and peptide effects are essentially the same in the *P* and *M* diastereomers, if the torsional angle change $C_\alpha H$–$C_\beta H_2$ can be disregarded. However, as we shall see, the disulfide influence should be very different in the two cases.

[4]) As the rotational strength resulting from the inherently asymmetric disulfide chromophore is at least one order of magnitude greater than that arising from electronic perturbations, a slight deviation from 90° could easily compensate an asymmetric influence of the diketopiperazine ring on the sulfur atoms of **1**.

The peptide group can be regarded as containing a partial double bond, OC(2)═══N(1), and thus **1** as bearing some similarity to the inherently asymmetric β,γ unsaturated ketones. The peptide group is in a negative octant according to the convention of the *Tinoco-Caldwell-Eyring* expression (1) [21] adopted here (or in a positive octant, following e.g. *Moffit et al.* [22] for ketones). A double bond in this octant of a β,γ unsaturated ketone would cause a positive *Cotton* effect (cf. the list in [23]). We can assume that the peptide group of **1** would behave similarly, leading to a (presumably weak) $(+)R_{n,\pi^*}$.

The influence of the methylene and disulfide groups on R_{n,π^*} can be semiquantitatively discussed with the help of the *Tinoco-Caldwell-Eyring* equation (1) [21]:

$$R_{n,\pi^*} = -\gamma_x \gamma_y \gamma_z \left[\frac{AQ + B\alpha}{r^4} + C\, f(z) \right] \qquad (1)$$

in which $\gamma_x \gamma_y \gamma_z$, the product of the direction cosines, determines the octant, A, B, and C are constants, Q, α, and $f(z)$ are the static charge, polarisability, and incomplete screening function for the perturbing group, and r is its distance from the carbonyl oxygen.

O(1) is perturbed by methylene $C(1)_\beta$ from a positive and by $C(2)_\beta$ from a negative octant. However $C(1)_\beta$ is closer (3.1 Å) than $C(2)_\beta$ (4.4 Å), leading to a ratio of magnitudes of $(+)R$ (from CH_2 in the negative) to $(-)R$ (from CH_2 in the positive octant) of approximately 0.25; the resulting *negative* Cotton *effect* is expected to have only about $^3/_4$ of the rotational strength it would have without $C(2)_\beta$.

With the substituent sulfur atoms, groups are introduced which have the same electronegativity as the methylene carbon atoms (thus not altering the net charge, Q, to any great extent) and produce an unknown, but certainly not too significant change in the incomplete screening functions. However, a large increase in polarisability, α, and, as a result, of $|R|$, is to be expected. This would appear to make R especially sensitive to the spatial disposition of the disulfide sulfur atoms with respect to the carbonyl oxygen. In the M diastereomer, the distances from O(1) to S(1) (+ octant) and to S(2) (− octant) are $\simeq$ 4.3 and 4.0 Å, respectively. We should expect a total $(+)R$ contribution of the disulfide group with partial contributions $(+)R$ (S(2)) and $(-)R$ (S(1)). The magnitude ratio $(-)R/(+)R$ can be calculated to be $\simeq$ 0.75. In the M configuration, the negative rotational strength resulting from the two methylene groups would thus be substantially decreased, or even become positive under the influence of the disulfide group, the change depending on the magnitude of the polarisability difference, $\Delta\alpha$.

The opposite should be the case for the P diastereomer. Here, the S(1) atom in the positive octant is closer than the S(2) atom in the negative (3.5 vs. 4.8 Å), resulting in a magnitude ratio $(+)R/(-)R \simeq 0.28$. A strong increase of *negative* rotational strength (about 72% of that introduced by the hemidisulfide residue in the positive octant alone) should result.

A comparison of the strong negative 228 nm *Cotton* effect of **1** with the known CD. of similar compounds suggests that we are actually dealing with the P helical form. In Fig. 9, the CD. of *cyclo*-L-alanyl-glycyl in ethanol is included [24]. In this compound, the alanyl methyl is in a positive octant with respect to the nearer, and in a

negative with respect to the more remote carbonyl oxygen atom. Assuming a planar conformation, the distances would be 2.8 and 4.5 Å, respectively, and the magnitude ratio $(+)R/(-)R \simeq 0.15$. On the basis of $R_{n,\pi^*} \simeq -1.5 \cdot 10^{-40}$ for *cyclo*-L-analyl-glycyl, a very approximate $R_{n,\pi^*} \simeq -1.8 \cdot 10^{-40}$ can be calculated for the influence of 1 methyl group in a positive octant at 2.8 Å from the carbonyl oxygen. Using this value as representing the methylene perturbation in diketopiperazines[5]) and excluding any factors resulting from rigidity, a very rough estimate of $R_{n,\pi^*} \simeq -2.7 \cdot 10^{-40}$ erg·cm³ for **1** can be made. Including rigidity, we would be inclined to assume a value of $R_{n,\pi^*} \simeq -10$ to $-20 \cdot 10^{-40}$ as probable[6]).

Thus, the disulfide group enhances the purely methylene induced rotational strength by about 15-fold to $R_{n,\pi^*} = -45.4 \cdot 10^{-40}$ without, and approximately 3-fold with correction for rigidity. An enhancement of similar magnitude has been observed for the stereochemically equivalent pair (*S*)-5-methyl-pyrrolid-2-one ($R_{208\,nm} = -6.5 \cdot 10^{-40}$ in trifluoro-ethanol) and (*R*)-5-iodomethyl-pyrrolid-2-one ($R_{212\,nm} = -35 \cdot 10^{-40}$ in dioxane; both the methyl and iodomethyl groups are in the same + octant) and explained in a similar manner by the increase of polarisability [25c].

4.5. *ORD.* The ORD. of **1** (Fig. 10) can be explained by the CD.: a large positive *Cotton* effect (CD. maximum at 198 nm) causes the general increase of $(+)[\Phi]$ from 400 nm on downwards. Its long-wavelength *extremum* is observed at $\simeq$ 200 nm (not shown in the Fig.). The steady rise is interrupted by superposition of the also relatively large negative *Cotton* effect located at 228 nm (CD.). In the ORD. only its long-wavelength *extremum* appears as a trough at 260 nm, the short-wavelength ORD. *extremum* being buried under the long-wavelength arm ($\simeq$ 200 nm) of the 198 nm *Cotton* effect.

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99. Effect of an External Electric Field on the Decay Constant of $^{99}Tc^{m}$ in Halogen Complexes

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Summary. A positive change $\Delta\lambda/\lambda$ of the decay constant of $^{99}Tc^{m}$ has been observed on the application of an external electric field in K_2TcX_6 (X = F, Cl, Br, I). For its investigation the Tc-complexes were embedded as small conglomerates or more or less as isolated single molecules in a powder of high dielectric constant, which was then compressed into a thin layer of about 0.2 mm and inserted between two capacitor plates. The change $\Delta\lambda/\lambda$ depends on the strength of the chemical bond of $^{99}Tc^{m}$. As a first approximation $\Delta\lambda/\lambda$ was assumed to be linearly dependent upon the electric field. The experimental results with an external field of 10^4 V/cm are:

Compound	K_2TcI_6	K_2TcBr_6	K_2TcCl_6	K_2TcF_6
$2\times10^5\ \Delta\lambda/\lambda$	6.1 ± 0.9	5.2 ± 0.7	5.9 ± 0.8	2.2 ± 0.9

A relationship between $\Delta\lambda/\lambda$ and the electro-negativities of the ligand atoms could be established. Difficulties encountered in the theoretical calculation of $\Delta\lambda/\lambda$ are discussed.

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