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174. On the Chirality of the Cystine Disulfide Group: Assignment of Helical Sense in a Model Compound with a Dihedral Angle Greater than Ninety Degrees using NMR. and CD.

by **Urs Ludescher** and **Robert Schwyzer**

Institut für Molekularbiologie und Biophysik, Eidgenössische Technische Hochschule Zürich 8049 Zürich

(21. VI. 71)

Zusammenfassung: Im synthetischen [2,7-Cystin]-Gramicidin S ist der Cyclodecapeptid-Ring mit einer Disulfidgruppe überbrückt. NMR.-Untersuchungen zeigen, dass die Peptidkette der Verbindung (wie beim Gramicidin S) in der antiparallelen β-Konstellation vorliegt. Auf Grund von Abschirmungs-Effekten kann der Disulfid-Brücke die *P*-helikale Chiralität zugewiesen werden. In Molekel-Modellen beträgt der dihedrale Winkel φ etwa 120°. Die *P*-helikale Anordnung, verbunden mit $|90°| < |\varphi| < |180°|$, bedingt nach der Theorie von *Linderberg & Michl* einen negativen Rotationsbeitrag der langwelligsten asymmetrischen Absorptionsbande, was mit unseren CD.-Versuchen nun erstmals experimentell bestätigt wird ($\lambda_{min} = 271{,}5$ nm, $\Theta = -7550$, Äthanol). Auch der theoretisch geforderte zweite *Cotton*-Effekt mit umgekehrtem Vorzeichen kann bei dieser Modellverbindung gut beobachtet werden ($\lambda_{max} = 230$ nm, $\Theta = +60870$, Äthanol); allerdings sind in diesem Bereiche Beiträge von Peptid-$n \rightarrow \pi^*$-Übergängen nicht *a priori* auszuschliessen, was den diagnostischen Wert dieser Bande vermindert.

Introduction. – The work of *Carmack & Neubert* and of other authors [1] has provided correlations between the chirality of the disulfide bond and the sign of the

two *Cotton* effects observed in the region of 250 to 300 nm and 230 to 250 nm, respectively, using model compounds of known asymmetry and with dihedral angles φ of $|0°| < |\varphi| < |90°|$. In this angle range (*cis*), the positive helical sense is related to a positive, at $\lambda > 250$ nm, and a negative *Cotton* effect at $230 < \lambda < 250$ nm (*vice versa* for a left-handed helical arrangement).

Based on the theoretical work of *Bergson* [2], *Linderberg & Michl* [3] have formulated a quadrant rule for the inherent optical activity of organic disulfides. Their results agree very well with the theoretical and experimental work of *Wagnière & Hug* [4] on the polarization and the sign of the long wavelength *Cotton* effects in general chromophores of symmetry C_2. It predicts that the signs of the *Cotton* effects in chiral disulfides with dihedral angles $|0°| < |\varphi| < 90°|$ (*cis*) will be the opposite of those in compounds with $|90°| < |\varphi| < |180°|$ (*trans*; Fig. 1). In the case of $\varphi = \pm 90°$, the

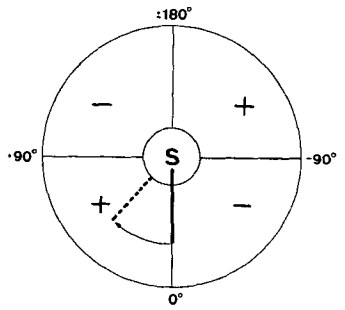


Fig. 1. *Quadrant rule for the inherent rotatory strength of the long-wave-length transition of the disulfide chromophore* (according to [2])

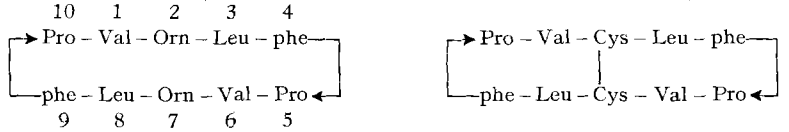
inherent optical activity should be zero despite the fact that the disulfide bond may be present in the *P*- or *M*-helical configuration[1]) (nomenclature see [5]).

[2,7-Cystine]-gramicidin S furnishes an example of a *P*-helical cystine disulfide group with effective symmetry C_2 and a *dihedral angle* $+90° < \varphi < +180°$. An example of a chiral cystine disulfide group with $\varphi = 90°$ will be discussed later [6]. These experimental results complete the evidence for the correctness of the theoretical predictions in both sets of quadrants.

The Secondary Structure of [2,7-Cystine]-Gramicidin S and the Chirality of the Disulfide Group – NMR. Studies. – Gramicidin S (**1**) is a homodetic [7] cyclic decapeptide with a rather stable secondary structure in solution (for references see [8]). In molecular models, the two ornithine residues in positions 2 and 7 can be replaced by two half cystine residues as in the homodetic-heterodetic, bicyclic

[1]) The inherent rotatory strength of an electronically unperturbed disulfide chromophore should be zero for $\varphi = 0°$ and $\pm 180°$ (effective symmetry C_2v and C_2h, respectively). For dihedral angles $\varphi = \pm 90°$, the first UV. absorption band is supposed to result from a superposition of two degenerate $\psi \rightarrow \sigma^*$ transitions with rotatory strengths of equal magnitude but opposite signs. Degeneracy is relieved if φ differs from 90°: two asymmetric transitions ($\psi_- \rightarrow \sigma^*$ and $\psi_+ \rightarrow \sigma^*$) with different energies and different signs should appear [3]. The signs of their rotational strengths are, in the case of effective C_2 symmetry, most probably related to the polarization of the transitions with respect to the symmetry axis (*A*- or *B*-symmetry of the transition) [4].

[2,7-cystine]-gramicidin S (**2**) without distorting the conformation of the polypeptide chain (Fig. 2). The disulfide group bridges the two β-antiparallel tripeptide strands and



1[2]) **2**

can be arranged with either *P*-helical or *M*-helical chirality and a dihedral angle φ of approximately $\pm 120°$[3]). In both cases, an axis of C_2 symmetry bisects the disulfide bond perpendicularly to the plane of the homodetic ring.

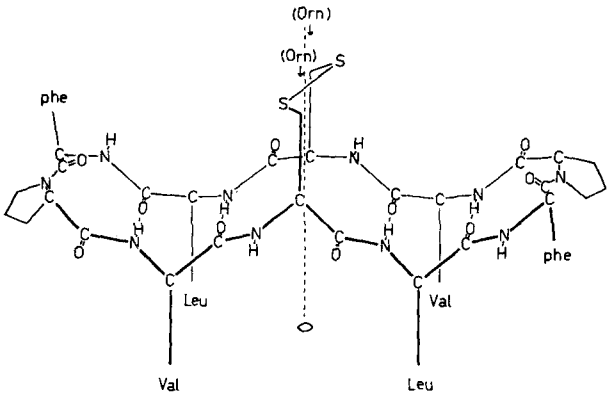
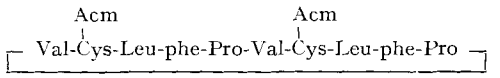


Fig. 2. *Schematic representation of the secondary structure of* **2** *as the* P*-helical diastereoisomer*
The side chains are identified by the amino-acid abbreviation, except for proline and cystine; replacement of the cystine side chain by two ornithine side chains gives **1**; the α-hydrogens have been omitted for clarity

We have synthesized pure crystalline [2,7-cystine]-gramicidin S from [2,7-bis-(S-acetamidomethyl-cysteine)]-gramicidin S (**3**) which had been obtained from the open chain decapeptide active ester **4** [9] by a synthesis analogous to that originally applied to gramicidin S [10].



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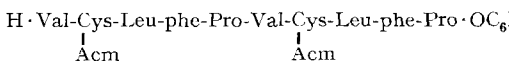

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The proton NMR. spectra of **2** and **3** determined in hexadeuteriodimethyl sulfoxide are almost identical with that of gramicidin S (**1**) except for the differences expected

[2]) Abbreviations according to the IUPAC-IUB rules, *e.g.* European J. Biochemistry *1*, 375 (1967); phe = D-phenylalanine; Acm = S-acetamidomethyl.

[3]) Clockwise rotation of the group remote from the observer (Fig. 1) or anticlockwise rotation of that which is nearer both produce *P*-helices: the dihedral angles φ are defined as *positive* in this disposition (this differs from the convention used by *Linderberg* & *Michl* [3], but appears to us to be apposite if used in connection with helical sense, *P* = plus, *M* = minus [5]).

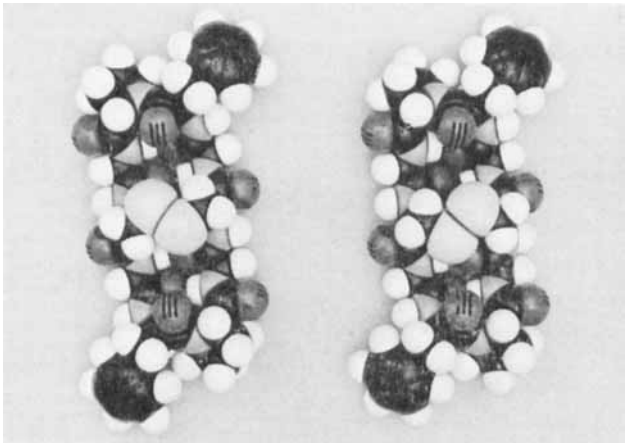

Fig. 3. *Space-filling models of [2,7-cystine]-gramicidin S viewed along the C_2 symmetry axis*
a) with *M*-helicity, b) with *P*-helicity of the disulfide bond. The side-chains of valine and leucine have been omitted for clarity

Proton NMR. resonances of gramicidin S (**1**), *[2,7-cystine]-gramicidin S* (**2**), *and [2,7-bis-(S-acetamidomethyl-cysteine)]-gramicidin S* (**3**) *in hexadeuteriodimethyl sulfoxide*

Number of protons	Correlations	Chemical shifts, ppm (J_{NC}, Hz) **1**	**2**	**3**
2	NH Phe	9.06 (~4)	9.03 (~4)	~8,9*)
2	NH Orn	8.66 (8.5)		
2	NH Cys		8.78 (8.5)	~8.78*)
2	NH Leu	8.33 (8.0)	8.34 (8.0)	8.38 (8.0)
2	NH Val	7.20 (8.5)	7.00 (8.5)	7.06 (8.5)
10	aromat. Phe	7.28	7.26	7.26
2	αCH Cys		5.3	4.9
2	αCH Orn	4.8		
2	αCH Leu	~4.6		
6	αCH Phe/Pro/Val	~4.4		
8	αCH Leu/Phe/Pro/Val		~4.4	~4.3
12	CH_2 (heteroatom/phenyl)	3.6/2.9	3.6/2.9	3.6/2.9
24	CH_2 and CH	1.5		
16	CH_2 and CH		1.5	1.5
24	CH_3	0.8	0.8	0.8
2	NH Acm			~8.6
4	CH_2 Acm			4.10
6	CH_3 Acm			1.90

*) Not as neatly separated as in **1** and **2**.

from the replacement of ornithine by cystine or S-acetamidomethyl cysteine (see table) [11]. The near identity of the observed chemical shifts and coupling constants ascribed to the C^{α} and N^{α} protons *strongly suggests identical secondary structures for all three compounds* (Fig. 2). There is one significant difference between the bridged compound **2** and gramicidin S (**1**): whereas the valine peptide protons appear at approximately 7.20 ppm in **1** (almost obscured by the phenyl proton signals), they are observed at 7.00 ppm in **2**. This degree of shielding of 0.2 ppm can be explained qualitatively by the vicinity of the sulfur 3*p*-orbitals if the disulfide bridge assumes *P*-helical chirality. These same orbitals are skewed away from the valine peptide protons in the *M*-helical diastereomer (Figs. 2, 3) and would be expected to shield the leucine peptide protons instead. However, there is no evidence for an upfield shift of these protons (see Table). *Hence, the NMR. data alone are strongly indicative of the* P-*helical chirality of the disulfide groups in all or the great majority of the molecules of [2,7-cystine]-gramicidin S in dimethyl sulfoxide solution.* There is evidence for like, but weaker, selective shielding in compound **3**, and of an extremely facile transformation into the disulfide [11], which might mean that in **3** and **2** the sulfur atoms are already occupying similar positions.

Circular Dichroism (CD.) of 1, 2, and 3. – The CD. spectrum of **3** (Fig. 4) in *ethanol* is very similar to that of gramicidin S (**1**) measured by *Quadrifoglio & Urry* [12]. Minima of molar ellipticity are observed at 205 nm (– 324,000), 217 nm (– 274,000)

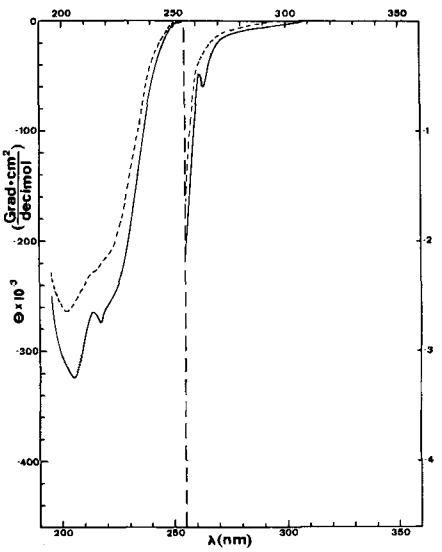

Fig. 4. *CD. spectra of [2,7-bis-(S-acetamidomethyl-cysteine)]-gramicidin S,* **3** in ethanol (–) and trifluoroethanol (– – –)

and, very weak, 263 nm (– 593), the last lying within the range of phenylalanine aromatic absorption [13]. In *trifluoro-ethanol*, this last minimum has not been detected, that at 217 nm appears only as a weak shoulder, and that at 205 nm is shifted to 202 nm (– 264,000).

The CD. of **2** is very different (Fig. 5): in *ethanol* two new minima are observed, one with a very broad band at 271.5 nm (−7730), and one very weak (artefact?) at 345 nm (−107). The negative peak at 205 nm (−445,000) retains its position but increases in intensity, whereas shoulders only are to be seen near 217 and 263 nm. *Trifluoroethanol* as solvent reduces the molar ellipticity by 20–30% and resolves a new band at 196 nm.

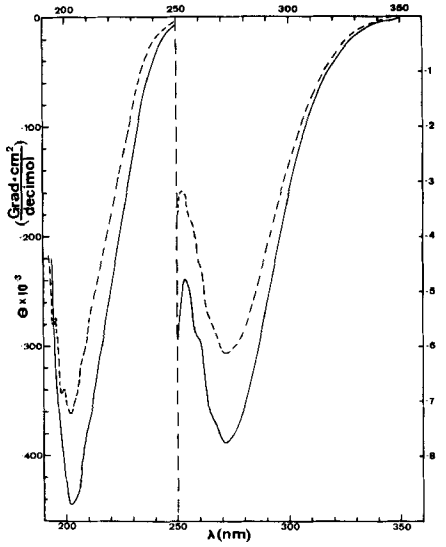

Fig. 5. *CD. spectra of [2,7-cystine]-gramicidin S,* **2** (solvents see Fig. 4)

Because of the identity of the secondary structure indicated by NMR., of all three compounds **1**, **2** and **3**, the spatial arrangement of the ten peptide chromophores relative to each other will be the same. Their total contribution to the CD. spectrum will also be equal, except for perturbations arising from the replacement of the ornithine side chains (positively charged ammonium groups) by the less polar and more polarizable S-acetamidomethyl-cysteine and cystine residues in **3** and **2**, respectively. A drastic change is certainly that from the strongly polar ornithine residue to the two less polar. It is difficult to foresee how great the differences of the perturbations (especially of the peptide $n \rightarrow \pi^*$ transitions) introduced by the different polarizabilities and spatial arrangements of the two sulfur-containing residues in **2** and **3** will be. However, their greatest influence will certainly become apparent at wavelengths shorter than approximately 240 nm[4]). We therefore believe that it is justifiable to calculate the difference spectrum $\Theta_\lambda(\mathbf{2})-\Theta_\lambda(\mathbf{3})$ as a first approximation to the inherent optical activity of our *P*-helical disulfide chromophore; this is shown in Fig. 6.

The difference spectrum in *ethanol* shows three negative *Cotton* effects at 271.5 nm (−7550), 211 nm (−87,500), and 202 nm (−126,000). A maximum is revealed at 230 nm (+60,870). In *trifluoroethanol*, a similar spectrum is observed: besides intensity

[4]) A similar compound, cyclocystine [14], shows no anomalies of the peptide chromophore above ~230 nm [6].

changes, there is a slight red shift of about 1 nm for the 271.5 nm band, and the positive band at 230 nm is displaced towards higher energies by about 5 nm. Whereas the 202 nm band remains at the same position, the 211 nm peak appears to be strongly displaced towards the blue, appearing at 196 nm (this assignment could be wrong, of course, for both bands could be shifted to the blue).

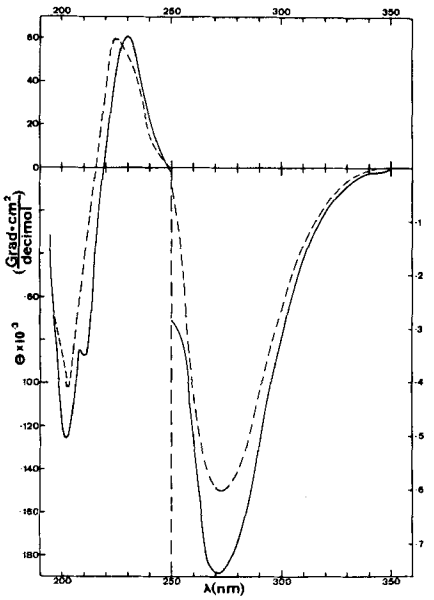

Fig. 6. *CD. difference spectra of* Θ_λ (**2**)–Θ_λ (**3**) (solvents see Fig. 4)

We suggest that the 271.5 nm band corresponds to the long-wavelength transition of the disulfide bond. If we take as correct a) the assignment of *P*-helical sense by NMR., and b) the conclusion that $\varphi \simeq +120°$ (measured in molecular models), then the observation of $\Theta_{271.5} = -7550$ confirms the rules of *Linderberg & Michl* [3] for the inherent optical activity of the disulfide chromophore. It also fits into the *Wagnière & Hug* [4] picture of the generalized asymmetric chromophore with effective symmetry C_2.

The 230 nm band (+60,870) certainly reflects the asymmetry of the higher energy, $\psi \rightarrow \sigma^*$, disulfide transition; it may, however, contain contributions from perturbed peptide $n \rightarrow \pi^*$ transitions. In any case – even though its positive sign is in keeping with theory – we would like to caution against its use as a diagnostic tool. The strong blue-shift by change of solvent from ethanol to trifluoroethanol suggests that it is particularly sensitive to surroundings [1] [2].

The rotatory strength, R, of the two transitions was approximated, using the relationship (1) [15]:

$$R = 1.234 \cdot 10^{-42} \cdot [\Theta^\circ] \frac{\Delta^\circ}{\lambda^\circ} \text{ (erg} \cdot \text{cm}^3\text{)}, \quad (1)$$

where λ° is the wavelength of the ellipticity peak, $[\Theta^\circ]$ is the ellipticity at this point, and Δ° is the half-width of the *Cotton* effect at $[\Theta^\circ] \cdot e^{-1}$. R was found to be quite strong and in the range typical for such 'inherently dissymmetric' chromophores [1] [2], *viz*: $-1.23 \cdot 10^{-39}$ erg · cm³ (271.5 nm) and $5.86 \cdot 10^{-39}$ erg · cm³ (230 nm). The value for the long-wavelength transition is in the order of 10 times that found for open-chain compounds (cystine and diacetyl-cystine-bis-methylamide) by *Coleman* & *Blout* [15].

The NMR. spectra were determined on a *Varian* XL100 Spectrometer by Dr. *Regula Keller* in the laboratory of Dr. *K. Wüthrich*. The CD. spectra were determined on a *Jouan-Roussel* Dichrograph placed at our disposal by Prof. *G. Wagnière* and Dr. *W. Hug* (Institute of Physical Chemistry, University of Zurich) whom we also thank for helpful discussions. Financial aid was obtained from the *Eidg. Stiftung zur Förderung Schweiz. Volkswirtschaft durch Wissenschaftliche Forschung* (Fellowship of *U.L.*), the *Swiss National Foundation*, and *CIBA-GEIGY Ltd.*, Basle.

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