192. Membrane Structure of Substance P

III. Secondary Structure of Substance P in 2,2,2-Trifluoroethanol, Methanol, and on Flat Lipid Membranes Studied by Infrared Spectroscopy[1])

by **Daniel Erne, Krzysztof Rolka,** and **Robert Schwyzer***

Institut für Molekularbiologie und Biophysik, Eidg. Techn. Hochschule Zürich, CH-8093 Zürich-Hönggerberg

(5.VIII.86)

IR data of the neuropeptide substance P (**1**) and its synthetic segments des-(Arg[1]-Gln[6])-substance P (**6**), des-(Arg[1]-Pro[4])-substance P (**4**), des-(Arg[1]-Lys[3])-substance P (**3**), and des-Arg[1]-substance P (**2**) indicate predominant β-structures in the solid state and α-helical structures in CF_3CH_2OH (amide I band shape analysis). In MeOH, the spectra of **1** suggest a partly helical structure. On membranes prepared from 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine, a C-terminal α-helix consisting of 8 or 9 peptide bonds appears to be induced (IR attenuated total reflection studies). Its perpendicular orientation on the membrane is suggested by the dichroic ratios of the amide-I and -II bands. This study is consistent with our CD experiments and lends support to the membrane structure of **1** predicted from the estimated amphiphilic moment, hydrophobic-association constant, and helix length.

1. Introduction. – Various groups have investigated the secondary structure of substance P (**1**) and its C-terminal segments (amino-acid sequences in *Table 1*). IR [1b] and CD [1b] [2] [3] studies provided no evidence for ordered structures in dilute aqueous solutions.

Table 1. *List of Peptides*[a])[b])

	1	2	3	4	5	6	7	8	9	10	11
Substance P (**1**)	Arg-	Pro-	Lys-	Pro-	Gln-	Gln-	Phe-	Phe-	Gly-	Leu-	Met-NH_2
Des-Arg[1]-substance P (**2**)		Pro-	Lys-	Pro-	Gln-	Gln-	Phe-	Phe-	Gly-	Leu-	Met-NH_2
Des-(Arg[1]-Lys[3])-substance P (**3**)				Pro-	Gln-	Gln-	Phe-	Phe-	Gly-	Leu-	Met-NH_2
Des-(Arg[1]-Pro[4])-substance P (**4**)					Gln-	Gln-	Phe-	Phe-	Gly-	Leu-	Met-NH_2
Des-(Arg[1]-Gln[5])-substance P (**5**)						Gln-	Phe-	Phe-	Gly-	Leu-	Met-NH_2
Des-(Arg[1]-Gln[6])-substance P (**6**)							Phe-	Phe-	Gly-	Leu-	Met-NH_2
7	Arg-	Pro-	Lys-	Pro-	OMe						

[a]) [Leu[9]]Substance-P peptides are analogues of their substance-P counterparts in which Gly-9 is replaced by Leu-9.

[b]) The residue numbering of **1** and [Leu[9]]-**1** is retained for their shorter peptide segments.

[1]) Peptide nomenclature and abbreviations, see IUPAC-IUB JBCN Recommendations 1983 on 'Nomenclature and Symbolism for Amino Acids and Peptides' [1a] and *Table 1*. Amino-acid residues are in their L-configuration unless explicitly stated otherwise. Random coil means nonregular, nonrepeating sequences of backbone dihedral angles with or without equilibria between various random-coil conformers. IR-ATR = infrared attenuated total reflection spectroscopy. POPC = 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine.

However, raising the concentrations of des-(Arg[1]-Lys[3])-substance P (**3**) to 0.1 mM and of des-(Arg[1]-Pro[4])- (**4**), des-(Arg[1],Pro[2])-, and des-Arg[1]-substance P (**2**) and **1** to 10 mM induced intermolecular aggregation. The aggregates consisted of highly ordered β-structures [1b] [3]. Studies of H/D exchange in peptide films showed that the C-terminal penta- to decapeptides possess a number of slowly exchanging amide protons, whereas the C-terminal tetrapeptide exchanges its amide protons very easily [1b]. In this series, **3** in which the positively charged N-terminal tripeptide is missing, has some noteworthy properties. Not only does its potency exceed that of **1** in certain assays [4] [5], but it also exhibits the highest tendency for intermolecular aggregation. Furthermore, its CD spectrum is time-dependent [3].

These studies were complemented by an IR investigation of the self-association of N(α)-protected C-terminal segments of **1** in mixtures of CH_2Cl_2 and DMSO [6]. Solvent-titration studies of the ^{1}H-NMR spectrum of Boc-Phe-Phe-Gly-Leu-Met-NH_2 (Boc-**6**) revealed that the amide protons of Phe-7, Phe-8, Met-11, and one proton of the primary amide group are strongly solvent-dependent, whereas the amide groups of Gly-9, Leu-10, and one proton of the primary amide group show reduced solvent accessibility (residue numbering of **1**). Self-aggregation is markedly reduced in [3-(2-aminoisobutyric acid)]-**6** (residue numbering of **6**). The two geminal CH_3 groups at C(α) are known to induce the formation of folded structures of the β-bend type [7] and, hence, to reduce aggregation to β-sheets.

A recent ^{1}H-NMR and CD study of the three-dimensional structure of substance P (**1**) is in agreement with the previous investigations as far as the conformations in H_2O are concerned [8]. As preferred structure in MeOH, the authors postulated a monomeric species stabilized by intramolecular H bonds. A model was proposed consisting of a random-coil N-terminal tripeptide segment, Arg-Pro-Lys-, an α-helical part, -Pro-Gln-Gln-Phe-Phe-, and a folded C-terminal segment, -Gly-Leu-Met-NH_2, in which the primary amide group interacts with the γ-carbonyl O-atoms of both glutamines (*Fig. 1a*). It

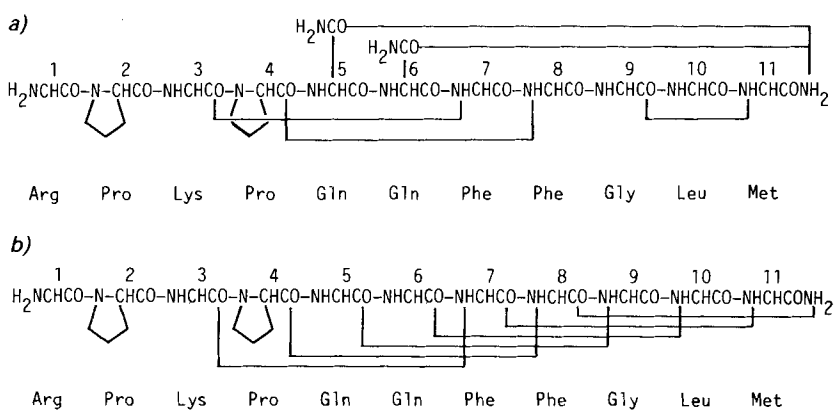

Fig. 1. *Proposed H-bonding systems for substance P. a)* The mixed α-helix/folded structure in MeOH of *Chassaing et al.* [8]. *b)* The α-helix with $n = 8$ –CO–NH– peptide bonds, one –CO–N= (Lys-Pro) peptide bond, and $m = 9$ amino-acid residues.

Except for proline, only the backbone atoms are shown. Horizontal and vertical lines represent H bonds between C=O and NH groups. The side-chain carbamoyl groups of Gln-5 and Gln-6 are indicated in *a)* to show their interaction with the C-terminal Met amide group.

resembles a folded structure with only one H bond from the side-chain NH_2 of Gln-6 to the C-terminal carbonyl O-atom of Met-11 suggested earlier and supposed to meet requirements of substance-P receptors [9].

To avoid problems with aggregation, **1** was bonded through its C-terminal methionine to polyethylene glycol monomethyl ether (PEG) [10]. From CD and IR, it was concluded that PEG-bonded molecules of **1** adopt predominantly random-coil conformations in CF_3CH_2OH, with indications of a β-turn located in the N-terminal region. The intrinsic tendency towards a folded structure appears more pronounced in the intermolecularly unaggregated, PEG-bonded peptide compared to native **1**. Influences of the proximity of peptide and carrier were not examined.

Our own CD studies [11] showed a significant increase of α-helix content with respect to random-coil structures of **1**, [Leu^9]-**1**, and their C-terminal segments (**2–4**, **6**, [Leu^9]-**2** to [Leu^9]-**4**, and [Leu^9]-**6**; see *Table 1*) when changing from an aqueous to a more hydrophobic environment in CF_3CH_2OH, a solvent known to favour ordered structures and to mimic the natural environment of membrane proteins [12]. Similar changes in the conformation of **1**, [Leu^9]-**1**, and **2** were observed in the presence of lipid vesicles prepared from phosphatidylserine [11]. Moreover, estimations of conformation, orientation, and accumulation of **1** and [Leu^9]-**1** on lipid membranes predict C-terminal α-helices comprising residues 3–11; these helices are oriented almost perpendicularly on the membrane surface [13].

Here, we investigate the conformation of substance P (**1**) and its C-terminal segments (including [Leu^9]-substance-P peptides) with IR and IR-ATR [14]. Band-shape analysis indicates predominantly helical structures for the C-terminal penta- and heptapeptides and partially helical structures for the deca- and undecapeptides with α-helices for residues 3–11 and more 'open' structures for residues 1–4, in agreement with the CD data and the estimations. IR-ATR suggests a similar partially helical structure of **1** on flat POPC membranes in which the helices are oriented about perpendicularly on the membrane surface. Thus, **1** behaves like dynorphin-A-(1–13)-tridecapeptide [15a] and adrenocorticotropin-(1–24)-tetradecapeptide [16] when imbedded into lipid membranes. The only difference is, that these peptides insert their more hydrophobic N-termini into the hydrophobic membrane environment, and substance P its more hydrophobic C-terminus.

2. Experimental. – Substance P (**1**) was purchased from *Bachem*, Bubendorf; all substance-P segments were synthesized by *K. R.* [15b]. Because of a strong interference of the carboxylate stretching vibration ($\bar{\nu}$(antisym.)) at 1610–1550 cm^{-1} with the amide-I and -II bands, the peptides were converted from their acetates to their respective HCl salts. CF_3CH_2OH was *Uvasol* grade from *Merck*, MeOH *(puriss., p.a.)* was from *Fluka*, Buchs.

IR spectra were recorded on a spectrophotometer *Perkin-Elmer 983G* equipped with a *PE* data station, model *3600*. Calculated spectra were plotted on a *PE* model *660* graphics printer. IR spectra of solid samples in KBr were obtained by mixing and compressing 0.3 to 1 mg of peptide with 300 mg KBr. Solution spectra were recorded in a CaF_2 cell (path length 0.025 mm) in the double-beam mode against air in the reference beam. The peptide spectra were then calculated by substraction of the pure-solvent spectra. IR-ATR spectra were obtained on a germanium total reflection element with an incident angle of 45°. For measurements of orientation, linearly polarized IR radiation, obtained with an AgBr grid polarizer, was used. Vertical (*vp*, 0°) means vertical, parallel (*pp*, 90°) means parallel with respect to the plane of incident radiation. Unless stated otherwise, the samples were equilibrated with a current of H_2O-saturated N_2 and dried at r.t. in a stream of dry gas before measurements. The spectra shown are usually averaged spectra of 16 to 64 scans. Peak positions were determined with a peak identification routine supplied by the *PE983* applications program. Derivative spectra (first and second derivatives) were calculated using 'obey' program routines to determine shoulders on absorbance peaks. For direct comparison of peaks of different compounds and/or concentrations, some of the spectra were expanded using the absorbance expansion 'abex' routine of the *PE983* applications program.

3. Results and Discussion. – When analyzing IR spectra of substance P (**1**) in the region of 1700–1500 cm^{-1}, a few things must be kept in mind. In this spectral region, the amide-I and -II bands may be overlapped by contributions of the Gln side-chain carbamoyl group (free 1690, associated 1650 cm^{-1}; amide-I band; free 1600, associated 1640 cm^{-1}; amide-II band) and of the Arg guanidinium group (1660 and 1630 cm^{-1}; monosubstituted guanidinium-I and -II bands). Contributions of the C-terminal -Met-NH_2 are considered to be comparable to those of Gln carboxamide. Interference of aromatic Phe side chains at 1600 and 1500 cm^{-1} may also be expected. (All wave numbers mentioned above are approximate and typical for the functional groups, they are not experimental values for the specific amino acids.) Similar problems arise when analyzing the CD spectra of substance-P peptides in their $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transition region. Particularly for rather short peptides, these effects are not to be neglected, see [11].

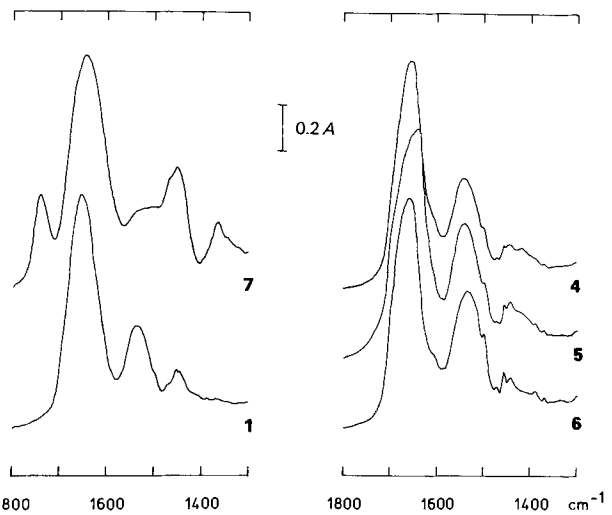

Fig. 2. *IR of* **1** *and some shorter segments in KBr.* For direct comparison, the spectra are expanded to 1 absorbance unit.

Fig. 2 shows the IR of substance-P peptides in the solid state (KBr discs). The amide-I band clearly indicates different secondary structures. Besides a main random and/or α-helical contribution, there is evidence for some kind of β-structure (shoulders at *ca.* 1698 and 1637 cm^{-1}). Peptide **5** has by far the greatest β-contribution. This is also reflected in the half-width of the amide-I band, which is significantly broader than for other peptides.

The spectra of **6** in KBr *(Fig. 2)* and on a Ge total reflection element *(Fig. 3)* are distinctly different. On a Ge plate, the β-structure is dominant, especially after equilibration in *ca.* 100% relative humidity and subsequent drying. The dichroic ratio R for amide-I (1636 cm^{-1}) and amide-II (1540 cm^{-1}) bands is 1.12 and 1.10, respectively. They do not deviate significantly from that expected for an isotropic sample as given by *Eqn. 1* [14],

$$R_{iso} = (E_x^2 + E_z^2)/E_y^2 \qquad (1)$$

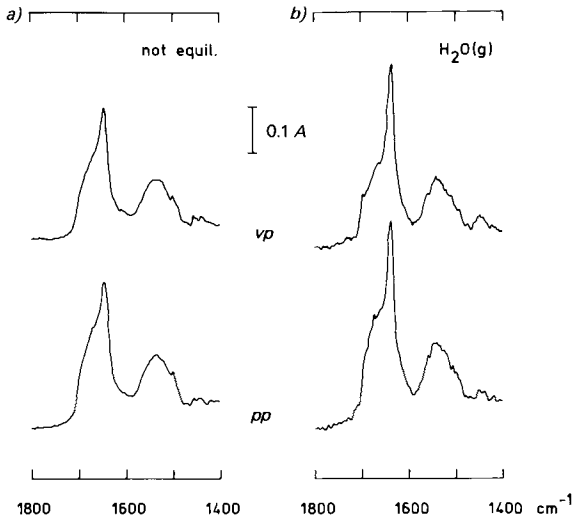

Fig. 3. *IR-ATR of* **6** *on a Ge reflection element. a)* Not equilibrated. *b)* After equilibration with H_2O vapor and subsequent drying.

where $E_{x,y,z}$ are the electric-field components in the directions of the principal axes of the coordinate system. With $n_1 = 4$ (Ge), $n_2 = 1.5$ (peptide), and $n_3 = 1$ (N_2), R_{iso} is 1.14. For polarizations of the absorptions at 1636 cm^{-1} (B_1 transition of amide I) and 1540 cm^{-1} (amide II) parallel and perpendicular to the C=O vector(s), respectively [17], the antiparallel β-pleated sheets of **6** appear to be lying flat on the solid surface.

IR data of substance-P peptides in CF_3CH_2OH solution are shown in *Fig. 4*. Sensitivity sets a limit to dilution below the millimolar range. Although **1** is strongly aggregated in

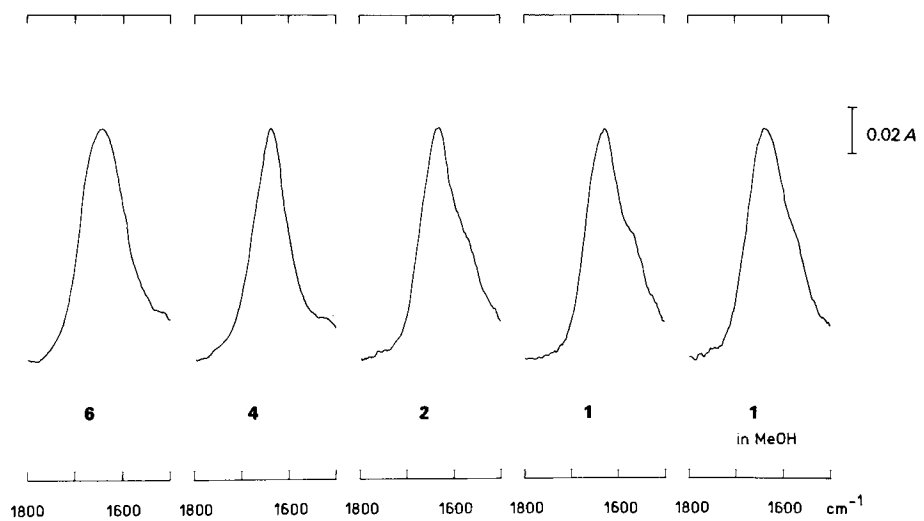

Fig. 4. *IR of* **1** *and some shorter segments in* CF_3CH_2OH *solution* (**6**, 24.3 mM; **4**, 12.4 mM; **2**, 6.0 mM; **1**, 3.8 mM) *and of* **1** *in MeOH solution* (3.5 mM). For direct comparison, the spectra are expanded to 0.1 absorbance unit.

CH_2Cl_2, aggregation is significantly reduced in H-bond-forming solvents such as pyridine, MeCN, DMF, DMSO, and MeOH [6] [8] [18]. CD of **1–4**, **6**, [Leu^9]-**1**, [Leu^9]-**3**, [Leu^9]-**4**, and [Leu^9]-**6** in CF_3CH_2OH and CF_3CH_2OH containing 25–75% H_2O provided evidence for α-helical monomers [11]. IR data typical of α-helix and/or 'parallel' β-turn were observed for **1** in CF_3CH_2OH [10], but an α-helix was excluded on the grounds that a shift of the amide-I absorption from 1650 to 1662 cm^{-1} *'can be explained by an increasing tendency of the peptide for aggregation in CF_3CH_2OH'* and that *'From data about the critical chain length of helix formation as well as from conformational-energy calculations, the onset of a helix structure in these short oligopeptides is very unlikely'* (no references) [10]. Our CD results with substance-P and [Leu^9] substance-P peptides indicating monomeric α-helical structures of short chain length [11] are explained by the observations of *Katakai* and *Iizuka* [19], who showed that the critical chain length for helix formation is strongly reduced for peptides containing groups with conformational destabilization such as glycine or a terminal charged group. In our peptides, both are present (**1–3**, [Leu^9]-**3** and [Leu^9]-**1** even contain prolines). The lack of the N-terminal charge may have caused the tendency of Boc-**6** to aggregate to β-structures in CH_2Cl_2 solution containing only small amounts of DMSO [6]. We therefore assume, in a first approximation, predominantly monomeric species in our CF_3CH_2OH solutions. The results show that this is reasonable.

Solutions in CF_3CH_2OH of **6**, **4**, **2**, and **1** give significantly different IR *(Fig. 4)*. Clearly distinguishable main-peak positions as well as distinct shoulders allow band-shape analysis by assuming the amide-I band to be composed of a set of overlapping *Lorentz*ian bands *(Eqn. 2)* [20],

$$A(\tilde{\nu}) = (w/\pi) \cdot A_0/[(\tilde{\nu}-\tilde{\nu}_0)^2 + w^2] \qquad (2)$$

where $A(\tilde{\nu})$ is the absorption, w the half-width [cm^{-1}], A_0 the peak area, and $\tilde{\nu}_0$ the wave number at peak absorption [cm^{-1}]. The number of components is set equal to the number of peaks and shoulders visible in the spectra. Since in CF_3CH_2OH we can expect some degree of ordering of the peptides [11] [12], we have tried to fit each main peak by a theoretically calculated amide-I band for a finite α-helix. This consists of a set of two or three components with the positions and relative intensities predicted for structures of different chain length [20]. In particular, helices with 5 peptide bonds (n = 5; 1679, 1664, and 1646 cm^{-1}; relative intensities 0.40, 0.50, and 0.10, resp.), 7 peptide bonds (n = 7, 1668, 1666, and 1650 cm^{-1}; relative intensities 0.70, 0.16, and 0.14, resp.), and 8 peptide bonds (n = 8; 1669 and 1656 cm^{-1}; relative intensities 0.67 and 0.33, resp.) are considered. *Fig. 5* shows two examples of fitted bands, and *Table 2* presents the results obtained for all spectra in CF_3CH_2OH. The parameters of the *Lorentz*ian bands chosen have been determined by visual observation of how well the calculated absorption band matches the experimental one. The values in *Table 2* are by no means thought to be the best and only ones possible. IR of [Leu^9]-**6**, [Leu^9]-**4**, [Leu^9]-**3**, and [Leu^9]-**1** in CF_3CH_2OH are practically indistinguishable from those of their substance-P counterparts, indicating the same helix lengths in both series (details not shown here).

Although the minor absorptions around 1685 and 1635 cm^{-1} can be attributed to folded β-structures, they are likely to have strong contributions from carbamoyl and guanidinium groups. No attempt has been made to interpret the minor bands in terms of structural elements. For the main peak, however, it is easily verified with molecular

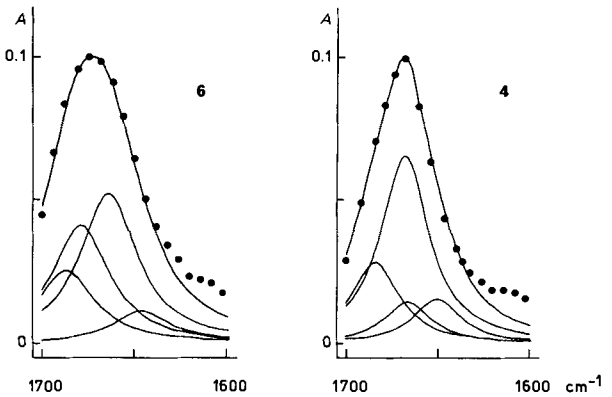

Fig. 5. *Band-shape analysis of the amide-I absorption of* **6** *and* **4** *in* CF_3CH_2OH *solution.* The spectra are normalized to 0.1 absorbance unit for direct comparison. Dots represent the experimental spectra *(cf. Fig. 3)*, solid lines are the single *Lorentz*ian bands and their sum.

Table 2. *Results of Band-Shape Analysis of the Amide-I Band of Substance-P Peptides*

Peptide	*Lorentz*ian components[a])			Remarks[b])
	position [cm^{-1}]	relative intensity	half-width [cm^{-1}]	
6	1687	0.25	38	19%
	1679*	0.41	38	
	1664*	0.52	38	α-helix, n = 5, 81%
	1646*	0.11	38	
4	1684	0.28	32	23%
	1668*	0.65	32	
	1666*	0.14	32	α-helix, n = 7, 77%
	1650*	0.15	32	
2	1683	0.15	25	8%
	1669*	0.65	31	α-helix, n = 8, 66%
	1656*	0.32	31	
	1635	0.28	42	26%
1	1682	0.12	25	6%
	1669*	0.65	32	α-helix, n = 8, 66%
	1656*	0.32	32	
	1633	0.32	42	28%
1 in MeOH	1682	0.22	30	13%
	1668*	0.62	38	
	1666*	0.13	38	α-helix, n = 7, 68%
	1650*	0.14	38	
	1635	0.24	40	19%

[a]) α-Helix components [20] are indicated by an asterisk. [b]) n = Number of –CO–NH– peptide bonds in the helix [20].

models that α-helices with 5, 7, 8, and 8 peptide bonds are conceivable for **6**, **4**, **2**, and **1**, respectively. These figures include the C-terminal carbamoyl group as peptide bond, but the Lys-Pro bond is not counted, as peptide bonds involving the secondary amino group of proline behave atypically in IR and are not included in the calculations of [20]. The H bonds stabilizing the proposed helical structures in hydrophobic environments are shown

in *Fig. 1*. The structure of **1** in CF_3CH_2OH, particularly the length of the α-helix and the presence of a non-helical N-terminal tripeptide domain, agrees with the estimated conformation of **1** on an aqueous-hydrophobic interface or on a lipid membrane [13].

Since the solution conformation of **1** proposed by *Chassaing et al.* [8] *(Fig. 1)*, was determined MeOH, it was interesting to compare the IR of **1** in CF_3CH_2OH and MeOH (*Table 2* and *Fig. 4*). In CF_3CH_2OH, the amide-I absorption is at lower energy and is slightly narrower than in MeOH. This may be explained by a lower degree of ordering in MeOH (shorter α-helix [20]) which agrees with our CD measurements [11].

In contrast to NMR, unambiguous determination of single peptide H bonds is impossible with IR. Neither is it possible to check the relative flexibility or ease of $^1H/^2H$ exchange of single functional groups. The same holds true for CD. Both methods combined and supplemented by an IR band shape analysis allow, however, postulation of the present model. Further insight into the mode of β-folding could be provided by analysis of the IR amide A bands (3500–3300 cm^{-1}) with solutions in $CHCl_3$ and CCl_4 [21]. This was prevented by the poor solubility of our compounds in these solvents.

Fig. 6 shows the IR of **1** on a Ge reflection element. The spectrum of the equilibrated undecapeptide resembles that of **6** *(Fig. 3)*. The absorption maximum is at 1639 cm^{-1}, most likely due to a β-structure.

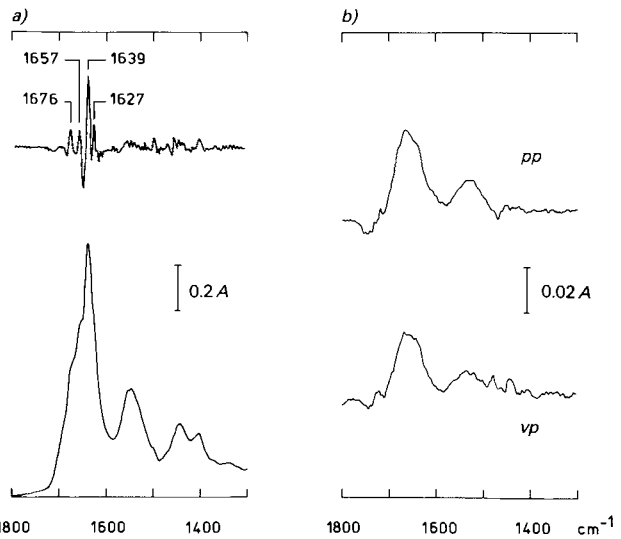

Fig. 6. *IR-ATR of* **1**. *a)* Spectrum obtained on a Ge reflection element (not polarized) and its negative second derivative spectrum (top). *b)* Polarized spectra obtained on POPC membranes (pure POPC membrane spectra subtracted). The molar ratio of lipid to peptide is 40. The spectra are recorded after equilibration with H_2O vapor and subsequent drying.

Polarized spectra on equilibrated POPC membranes are shown in *Fig. 6*. Maxima appear at 1666 cm^{-1}, and distinct shoulders at *ca.* 1686 and 1640 cm^{-1}. The apparent dichroic ratio for amide I ($R \approx 1.23$) is significantly different from the dichroic ratio of isotropic samples ($R_{iso} = 1.14$, *Eqn. 1*), whereas that for amide II ($R = 1.18$) is insignificantly different. Although the determination is subject to some uncertainties, probably

because of the disturbance by the side-chain carbamoyl and guanidinium groups, the dichroic ratios may be regarded as not only not contradicting, but even indicating a perpendicularly (±30°) oriented α-helical domain on the POPC membranes. This again agrees with the estimated conformation and orientation of **1** on a hydrophobic interface or on lipid membranes [13]. The result has nothing to do with the estimated association of **1** with neutral lipid membranes, which is very low, because in the preparation for IR-ATR, the peptide is precipitated onto the plate together with the lipid and cannot escape during the equilibration process. It can only become ordered and oriented.

4. Conclusions. – One of the problems in determining the conformation of substance-P and [Leu^9] substance-P peptides with IR and CD methods is the rather strong interference of side-chain carbamoyl, guanidinium, and aromatic groups with the contributions of the backbone peptide bonds. The problem is reasonably solved for the conformational aspect by a combination of CD [11] and IR: the results are consistent with and support the predicted membrane structure of **1** [13]. The aspect of orientation of the observed α-helix of **1** on flat membranes can not be answered in such a clear-cut manner, because the relevant dichroic ratios are smaller than for dynorphin-A-(1–13)-tridecapeptide [15a] and adrenocorticotropin-(1–24)-tetracosapeptide [16], probably because of interference by contributions of the randomly oriented side-chain groups to the spectra. However, the dichroic ratios are compatible with the calculated almost perpendicular orientation of the helix axis on the membrane surface ('perpendicular' means *ca.* 90° ± 30° in this context).

In the solid state in KBr, **1** and **4**–**7** appear to contain mainly randon-coil structures. After drying solutions of **6** on the surface of Ge plates, predominant antiparallel β-pleated sheets lying flat on the plate are observed. Substance P (**1**) also adopts a β-structure.

Band-shape analysis using theoretical parameters [17] [20] indicates mainly α-helical conformations in CF_3CH_2OH solution for substance-P and [Leu^9]substance-P peptides. The percentage of α-helix contribution to the total amide-I band is high in the short peptides **4** and **6** and involves all the possible peptide bonds plus the C-terminal carbamoyl group. A lower percentage of helix in **6** (77%) than in **4** (81%) may reflect the contributions of the Gln side-chain carbamoyl groups which increase the unidentified contributions to the amide-I band in **6**. For **1** and **2** in CF_3CH_2OH, 66% α-helix and helix lengths of 8 peptide bonds are found (see *Fig. 1*). The helix may well involve the C=O group of Lys 3, as postulated by the estimated structure [13] and by model construction; because of the atypical spectral behaviour of the Lys-Pro bond in its helical conformation, it may have escaped detection in the band-shape analysis. Helix percentage in **1** indicates a ratio of 8–9 helical peptide bonds (including the C-terminal carbamoyl group) to a total of 13 peptide and carbamoyl groups. This is in reasonable agreement with the postulated structure *(Fig. 1)*.

In MeOH, a somewhat shorter helix of **1** (7 peptide bonds) is observed. The difference to the results of *Chassaing et al.* [8] (4 peptide groups in the helix) is unexplained.

On POPC oligo-bilayer membranes equilibrated with N_2 saturated with H_2O, a definitely helical structure of **1** is indicated by IR-ATR. This result agrees with our CD measurements with liposomes [11] and with the estimated conformation of **1** (and [Leu^9]-**1**) on an aqueous-hydrophobic interface [13]. An almost perpendicular orientation of the helix axes on the membrane surface is suggested, again in agreement with the estimated orientation.

REFERENCES

[1] a) IUPAC-IUB JBCN, *Pure Appl. Chem.* **1984,** *56,* 595; b) B. Mehlis, S. Böhm, M. Becker, M. Bienert, *Biochim. Biophys. Acta* **1975,** *66,* 1447.

[2] B. Mehlis, M. Rueger, M. Becker, M. Bienert, H. Niedrich, P. Oehme, *Int. J. Peptide Protein Res.* **1980,** *15,* 20.

[3] M. Rueger, M. Bienert, B. Mehlis, K. Gast, D. Zirwer, J. Behlke, *Biopolymers* **1984,** *23,* 747.

[4] J. Bergmann, M. Bienert, H. Niedrich, B. Mehlis, P. Oehme, *Experientia* **1974,** *30,* 401.

[5] S. Caranikas, J. Mizrahi, E. Escher, D. Regoli, *J. Med. Chem.* **1982,** *25,* 1313.

[6] C. Toniolo, G. M. Bonora, G. Stavrapoulus, P. Cordopatis, D. Theodoropoulus, *Biopolymers* **1986,** *25,* 281.

[7] B. V. Venkataram Prasad, P. Balaram, *CRC Crit. Rev. Biochem.* **1984,** *16,* 307.

[8] G. Chassaing, O. Convert, S. Lavielle, *Eur. J. Biochem.* **1986,** *154,* 77.

[9] B. E. B. Sandberg, L. L. Iversen, *J. Med. Chem.* **1982,** *25,* 1009.

[10] P. Koziej, M. Mutter, H.-U. Gremlich, G. Hölzemann, *Z. Naturforsch., B* **1985,** *40,* 1570.

[11] K. Rolka, D. Erne, R. Schwyzer, *Helv. Chim. Acta* **1986,** *69,* 1798.

[12] T. P. Pitner, D. W. Urry, *J. Am. Chem. Soc.* **1972,** *94,* 1399.

[13] R. Schwyzer, D. Erne, K. Rolka, *Helv. Chim. Acta* **1986,** *69,* 1789.

[14] U.-P. Fringeli, H. H. Günthard, in 'Membrane Spectroscopy', Ed. E. Grell, Springer Verlag, Berlin-Heidelberg, 1981, pp. 270–332.

[15] a) D. Erne, D. F. Sargent, R. Schwyzer, *Biochemistry* **1985,** *24,* 4261; b) K. Rolka, R. Schwyzer, in preparation.

[16] H.-U. Gremlich, U.-P. Fringeli, R. Schwyzer, *Biochemistry* **1983,** *22,* 4257; *ibid.* **1984,** *23,* 1808.

[17] Yu. N. Chirgadze, N. A. Nevskaya, *Biopolymers* **1976,** *15,* 607.

[18] C. Poujade, S. Lavielle, G. Chassaing, A. Marquet, *Biochem. Biophys. Res. Commun.* **1983,** *114,* 1109.

[19] R. Katakai, Y. Iizuka, *Biopolymers* **1984,** *23,* 1411–1422; R. Katakai, *J. Am. Chem. Soc.* **1977,** *99,* 323–234; *Chem. Lett.* **1980,** 909.

[20] N. A. Nevskaya, Yu. N. Chirgadze, *Biopolymers* **1976,** *15,* 637.

[21] C. Pulla Rao, R. Nagaraj, C. N. R. Rao, P. Balaram, *Biochemistry* **1980,** *19,* 425.