191. Membrane Structure of Substance P

II. Secondary Structure of Substance P, [9-Leucine]substance P, and Shorter Segments in 2,2,2-Trifluoroethanol, Methanol, and on Liposomes Studied by Circular Dichroism[1])

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Comparative CD studies with substance P (**1**), [Leu^9]substance P ([Leu^9]-**1**), and their shorter peptide segments supported the membrane structures predicted for substance P and [Leu^9]substance P. They indicated that the C-terminal segments (from residue 3 or 4 onward) can adopt α-helical conformations in hydrophobic environments and on lipid membranes. The N-terminal segment, (residues 1–4) had a poly(proline)-like conformation in aqueous and hydrophobic surroundings. Residues 3 and 4 (Lys-Pro) appeared to belong to both domains and bring about the transition between the two. The estimated free energies of transfer for **1** and [Leu^9]-**1** from their random conformations in H_2O to their partially helical conformations on an aqueous-hydrophobic interface are too small to allow detectable interaction with neutral lipid membranes at low concentrations. The two peptides should, however, interact detectably with anionic membranes because of favourable *Boltzmann* distribution factors. This prediction was shown to be correct for liposomes prepared from 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (neutral) and phosphatidylserine (anionic).

1. Introduction. – Prediction of preferred conformation, orientation, and accumulation of substance P(**1**) and [Leu^9]substance P ([Leu^9]-**1**) *(Table 1)* on anionic lipid membranes [1b] calls for a detailed study of the secondary structure of **1** in surroundings

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^6)-substance P (**5**)							Phe-	Phe-	Gly-	Leu-	Met-NH_2
6								Phe-	Gly-	OEt	
7	Arg-	Pro-	Lys-	Pro-	OMe						

[a]) [Leu^9]-**1**, [Leu^9]-**3**, [Leu^9]-**4**, and [Leu^9]-**5** are analogues of the corresponding substance P peptides 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. PC = phosphatidylcholine; DOPC = dioleoyl-*sn*-glycero-3-phosphocholine; PS = phosphatidylserine (brain).

that mimic the membrane environment. In the following paper [2], we use IR and IR-ATR as conformation and orientation probes. Here, we studied **1**, [Leu^9]-**1**, and their shorter segments with circular dichroism (CD) in the membrane-mimicking solvent CF_3CH_2OH [3], in MeOH [4], and on liposomes prepared from zwitterionic DOPC and from anionic PS.

Previous CD studies indicated random-coil structures of substance P (**1**) and shorter segments in H_2O [5]. Aggregation of substance-P peptides in H_2O at concentrations above *ca.* 1 mM caused ellipticity extrema between 199–204 nm ($[\theta]_r = -10000$ to -21000[2])) and around 227–230 nm ($[\theta]_r = -1000$ to -7300[2])) suggesting 'B-type'-ordered structures [6]. Substance P and its shorter C-terminal peptides attached covalently to polyethylene glycol monomethyl ether (to prevent aggregation) were studied in CF_3CH_2O. With increasing chain length, two strong negative bands developed at 204 and 223 nm (see **1**-PEG in *Table 2*). They were interpreted as indicating a conformational transition from an unordered form to a β-turn in the N-terminal region (Arg^i-Pro^{i+1}-Lys^{i+2}-Pro^{i+3}-), leaving

Table 2. *Comparison of CD Extrema of Poly(amino acids) and Peptides*

Peptide	N[a])	Extrema[b])		Ellipticity ratio[c])	Solvent	Lit.
		$\lambda([\theta]_r)$	$\lambda([\theta]_r)$			
Poly(lysine)	900	208 (–32600)	222 (–35700)	0.91	H_2O[d])	[16]
Poly(lysine)	200	206 (–17800)	220 (–17100)	1.04	H_2O[d])	[e])
Poly(lysine)	55	205 (–19000)	220 (–15700)	1.21	H_2O[d])	[e])
H-(Leu-Leu-Ala)$_n$-OEt[f])	12	199 (–14000)	221 (sh, –5500)	2.55	$(CF_3)_2CHOH$	[22]
H-(Leu-Leu-Ala)$_n$-OEt[f])	15	201 (–16000)	221 (sh, –7300)	2.19	$(CF_3)_2CHOH$	[22]
H-(Leu-Leu-Ala)$_n$-OEt[f])	18	208 (–18500)	221 (–9500)	1.95	$(CF_3)_2CHOH$	[22]
H-(Met-Met-Leu)$_n$-OEt[g])	12	202 (–10000)	224 (–4800)	2.08	$(CF_3)_2CHOH$	[22]
H-(Met-Met-Leu)$_n$-OEt[g])	15	206 (–15000)	222 (–10800)	1.39	$(CF_3)_2CHOH$	[22]
H-(Met-Met-Leu)$_n$-OEt[g])	18	207 (–24500)	222 (–16400)	1.49	$(CF_3)_2CHOH$	[22]
1	11	205 (–4900)	222 (–3700)	1.32	PS[h])	[8]
1-PEG[i])	11	204 (–15500)	223 (–11370)	1.36	CF_3CH_2OH	[7]
Gramicidin S	10	206 (–32000)	218 (–30000)	1.07	H_2O	[20]
Cyclo(-Ala-Ala-Ahx-)[j])	3	205 (–5200)	220 (–6800)	0.76	$(CF_3)_2CHOH$	[21]
[Leu^9]-**1**	11	208 (–5600)	222 (–5000)	1.12	CF_3CH_2OH	[e])
1	11	205 (–3500)	222 (sh, –1900)	1.84	CF_3CH_2OH	[e])
2	10	205 (–5000)	222 (–4200)	1.19	CF_3CH_2OH	[e])
[Leu^9]-**1**	11	207 (–5200)	221 (–4800)	1.08	PS[k])	[e])
1	11	204 (–5200)	221 (sh, –3200)	1.63	PS[k])	[e])
2	10	207 (–2200)	221 (–2700)	0.81	PS[k])	[e])

[a]) N = number of amino-acid residues, approximate for poly(lysine).
[b]) λ in nm; for $[\theta]$, see *Footnote 2*.
[c]) Ellipticity ratio = short wavelength/long wavelength ellipticity.
[d]) pH 11.1.
[e]) This report.
[f]) HCl salt with n = 4, 5, and 6.
[g]) HCl salt with n = 4, 5, and 6.
[h]) Detergent-solubilized PS.
[i]) C-Terminal amide replaced by ester bond to poly(ethyleneglycol) monomethyl ether.
[j]) Allegedly in a type-I (or -III) β-bend conformation.
[k]) PS liposomes in 10 mM phosphate buffer, pH 6.4.

[2]) $[\theta]_r$ = mean residue ellipticity in degree·cm^2·dmol^{-1}.

the C-terminal part as random coil [7]. From a combined CD/NMR study of **1** [4], a specific secondary structure in MeOH was proposed. Three broad negative bands were observed at *ca.* 208, 220, and 230 nm. They were interpreted as indicating a partially α-helical structure. This was confirmed by NMR, and the α-helix was shown to comprise residues 4–8 (-Pro-Gln-Gln-Phe-Phe-). This stretch is preceded by a flexible Arg-Pro-Lys-domain, and followed by a 'U-turn' at Leu-10 which allows interaction of the C-terminal carboxamide with the primary amides of both glutamines. Furthermore, in 30 mM aqueous sodium dodecyl sulfate solution, **1** showed a similar CD as in MeOH, but with stronger negative ellipticity at 223 nm.

The influence of PC and PS dissolved in aqueous solutions of dodecyl heptakis(oxyethylene) ether or hexadecyl poly(oxyethylene) ether on the CD of **1** was investigated by *Wu* and coworkers [8]. At a lipid/peptide molar ratio of 1.6, PC did not change the spectra of **1** in H_2O. This was attributed to repulsion of the cationic peptide by 'positive charges on the polar head of the lipid'. PS, however, has a strong influence on the CD of **1**, inducing spectra that are indicative of partial helix formation (see *Table 2*). The authors explained this with attraction of the cationic peptide by the anionic head group of PS. These observations agree with results of *Lembeck* and coworkers [9] who showed that **1** readily partitions from aqueous buffers into solutions of PS in $CHCl_3$/MeOH, but not into solutions of PC at pH 7.2. The partition coefficient is strongly influenced by the charges on the lipid head group and on the peptide.

2. Material and Methods. – Peptides **1**–**7** *(Table 1)* were synthesized by classical methods in solution with purification and analysis of intermediates and products [10]. Solutions were prepared from the carefully dried HCl salts by weight; their molarity was not checked spectroscopically (lack of Trp and Tyr residues). CD was measured at r.t. with a *Jasco-J-500A* spectropolarimeter, equipped with a data processor *CD*, model *DP-500*. The results were plotted as the mean residue ellipticity $[\theta]_r$ [degree·cm^2·dmol^{-1}] [11] against the wavelength λ [nm]. CF_3CH_2OH was spectroscopic quality, 10 mM phosphate buffer was prepared from *puriss.* reagents with twice distilled H_2O and adjusted to pH 6.4. DOPC and PS were commercial products and were checked for purity (TLC, IR).

Liposomes were prepared according to [12] as follows: Phospholipid (5 mg) was dissolved in $CHCl_3$ (0.5 ml). The solvent was evaporated in a stream of N_2 and the sample dried at 11 Torr for 2 h. Phosphate buffer (0.5 ml) was pipetted onto the lipid film, and the lipid dispersed, first with a *Vortex* mixer, then by sonication in a bath sonicator *(Bransonic 221)* during 15 min. The clear liposome soln. (see [13]), was added to an appropriate quantity of peptide soln. (usually 0.8 mM) in phosphate buffer. The mixture was left at r.t. for 10 min, and buffer was added to adjust the peptide to the required molarity. Usually, 8 scans of CD were run. The spectra of equally dilute liposome solns. without peptide were subtracted.

3. Results and Discussion. – 3.1. *Phe and Pro Peptides in H_2O and CF_3CH_2OH* (see *Fig. 1*). In H_2O, Phe-Gly-OEt (**6**) has one positive extremum at 217 nm. Its position remains unaltered in CF_3CH_2OH, but its ellipticity is reduced by 1500 units to 85%. Phe-Phe-NH_2, substance P (**1**), and related peptides with Phe residues have positive bands in the same region which are attributed to a superposition of amide $n\rightarrow\pi^*$ and benzyl ^1L_a transitions [5]. With tetrapeptide **7** in H_2O, one strong negative extremum is observed at 204 nm. Again, the minimum is only slightly shifted in CF_3CH_2OH and its ellipticity reduced by 1400 units to 82%. Helical poly(proline) in CF_3CH_2OH [14] and pivaloyl-Pro-Val-NH_2 in its random structure in protic solvents [15] have CD almost identical with that of **7** (one strong negative extremum at 205 nm). CD indicates that **7** has the same conformation in H_2O and CF_3CH_2OH, thus behaving like pivaloyl-Pro-Val-NH_2. Its conformation may resemble both the 'random' structure of pivaloyl-Pro-Val-NH_2 and

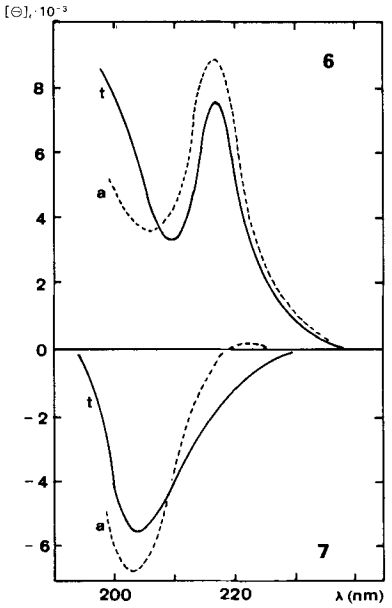

Fig. 1. *CD of* **6** *and* **7** *in* H_2O (---; $1 \cdot 10^{-4}$ and $2 \cdot 10^{-4}$ M, respectively) *and in* CF_3CH_2OH (——; $1 \cdot 10^{-4}$ M each)

the ordered structure of the poly(proline) helix. To interpret the CD of substance P and [Leu^9]substance-P peptides, the two bands at 204 and 217 nm must be taken into account. Where only the Phe residues are present, strong interference by the positive benzyl band in the diagnostic 210–225 nm region is to be expected. Where both structures **6** and **7** are present, additional interference around 200–210 nm should be anticipated.

3.2. *C-Terminal Peptides* **3**–**5** *and their [Leu^9] Analogues in* H_2O *and* CF_3CH_2OH (see *Fig. 2*). In H_2O, the influence of the benzyl groups is dominant, with positive extremes near 217–220 nm. Otherwise, the curves are indicative of random coil structures [5] [10] [15]. In CF_3CH_2OH, an increase of ordered structures with chain length is indicated. The negative extremum below 200 nm is shifted to longer wavelengths, and negative ellipticity develops in the 210–230-nm region. The ordering is already seen with **5** and [Leu^9]-**5**, where the intensity at 217 nm decreases to 21% (**5**) and 44% ([Leu^9]-**5**) on changing the solvent from H_2O to CF_3CH_2OH. This is significantly more than the solvent effect observed with **6**. The CD curves of **3** and **4** are explained by the presence of random coil and α-helical structures. Thus, the positions, relative intensities, and shapes of the extremes and shoulders of the curves of **3** and **4** correspond closely to those estimated by *Greenfield* and *Fasman* [16] for a mixture of 80% random coil and 20% α-helix of poly(lysine). it is not necessary to include β-structures to explain the curves of these peptides in CF_3CH_2OH. Accounting for the influence of the benzyl groups may further reduce the mean residue ellipticities at 222 nm of **5** by *ca.* 2400, and of **3** and **4** by *ca.* 1400–1500 degree·cm^2/dmol. We, therefore, conclude that 20% α-helix is a minimal value, and that the helix content may be considerably greater. This proved to be the case, as shown by analysis of the IR amide I absorption bands [2].

The trend towards α-helical structures is more pronounced with [Leu^9]substance P peptides. In this series, Gly-9, a strong 'helix breaker' and 'β-turn former' [17], is replaced

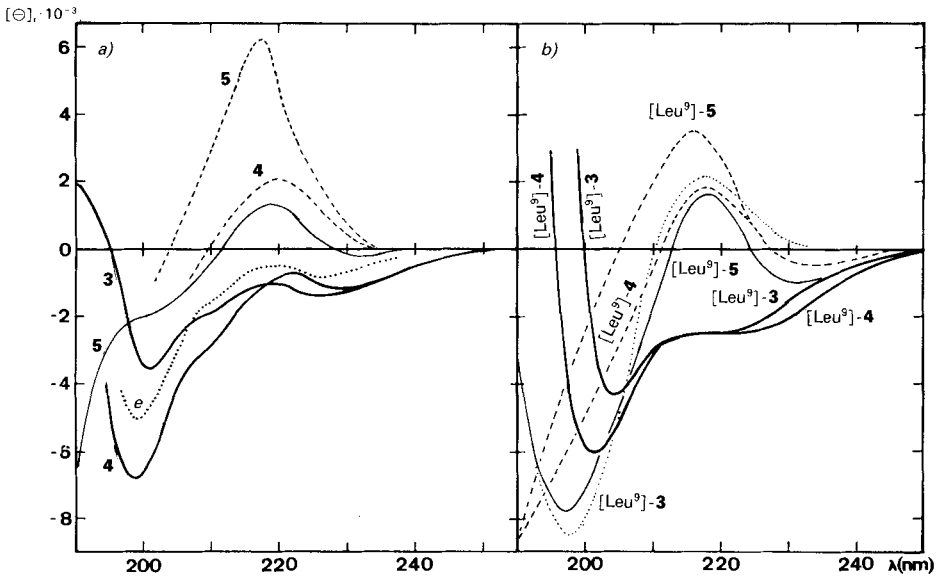

Fig. 2. *CD of substance P and [Leu⁹]substance-P peptides.* a) **5** ($4 \cdot 10^{-5}$ M) and **4** ($1 \cdot 10^{-4}$ M) in H_2O (----); **5** ($2 \cdot 10^{-4}$ M), **4** ($5 \cdot 10^{-4}$ M), and **3** ($2 \cdot 10^{-4}$ M) in CF_3CH_2OH (——). Curve *e* (· · · · ·) is the CD estimated for a mixture of 80% random coil and 20% α-helix [16] normalized to $[\theta]_\lambda = -5000$ at its 199 nm extremum. b) [Leu⁹]-**5** ($4 \cdot 10^{-5}$ M), and [Leu⁹]-**4** ($4 \cdot 10^{-5}$ M) (– – –), and [Leu⁹]-**3** ($8 \cdot 10^{-5}$ M; · · · ·) in H_2O; [Leu⁹]-**5** ($4 \cdot 10^{-4}$ M), [Leu⁹]-**4** ($2 \cdot 10^{-4}$ M), and [Leu⁹]-**3** ($2 \cdot 10^{-4}$ M) in CF_3CH_2OH (——).

by Leu, a strong 'helix former', a moderate 'β-sheet former', and a 'β-turn breaker'. Leu is expected to stabilize helices in hydrophobic surroundings by *ca.* 10 kJ/mol more than Gly [18]. CD curves of [Leu⁹]-**3**, [Leu⁹]-**4**, and [Leu⁹]-**5** in CF_3CH_2OH hardly differ from those of **3**, **4**, and **5**, respectively, except for the stronger intensity in the 215–225-nm region. Comparison with the computed curves [16] shows contributions of random coil and α-helical structures. Thus, [Leu⁹]-**3** might contain *ca.* 50–60% random coil and 40–50% α-helix. If the benzyl contribution is accounted for, a much larger proportion of α-helix appears probable. Mixtures with β-structures are excluded on the basis of the computed curves. β-Turns of the gramicidin-S [19] type have negative CD bands at *ca.* 206 and 218 nm [20], (see *Table 2*). Such a similarity between the CD of α-helical and cyclic peptides *(Table 2)* is also observed and discussed by *Bandekar et al.* [21]. However, reverse turns of this type in our Leu-9 peptides seem rather improbable [17].

A more relevant similarity exists between the CD of our C-terminal peptides in CF_3CH_2OH and short helical peptides in $(CF_3)_2CHOH$. *Katakai* and *Iizuka* [22] examined HCl·H-(Leu-Leu-Ala)$_n$-OEt and HCl·H-(Met-Met-Leu)$_n$-OEt with $n = 3$–6 in $(CF_3)_2CHOH$. With increasing chain length, the negative extremum around 200 nm is shifted to longer wavelengths *(Table 2)*. With the Leu peptides, a shoulder, and with the Met peptides, a negative peak developed in the 220–225-nm region. The ellipticity ratio (short wavelength/long wavelength ellipticity) decreased with chain length *(Table 2)*. The authors conclude that α-helices may be formed from the dodecapeptides onward, but that the critical chain length for α-helix formation may be shorter in *'peptides containing groups with conformational destabilization, such as glycine, or a terminal charged group'*.

Our peptides all have an N-terminal charge and a glycine residue; moreover, **1**, **2** and [Leu^9]-**1** have additional destabilizing residues: proline.

Table 2 shows that the intensities of the extrema usually assigned to the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ helix transitions tend to decrease quite generally with decreasing chain length of the helical peptides. A concomitant increase of the ellipticity ratio of the two bands is also seen.

We conclude that our C-terminal substance-P [Leu^9]substance-P peptides in CF_3CH_2OH increasing amounts of α-helical conformers in equilibrium with random coil conformers, and helices of increasing length in the penta-, hepta-, to octapeptide series. This view was fully supported by IR studies [2]: amide-I bands-shape analysis indicates 5 helical peptide bonds (including the C-terminal carbamoyl group) in **5** that account for 81% of the total contributions to the amide-I band. For **4**, the figures are 7 helical peptide bonds responsible for 77% of all contributions.

3.3. *[Leu^9]-***1**, **1**, *and* **2** *in H_2O, MeOH, and CF_3CH_2OH* (see *Fig. 3*). The CD curves of the three peptides in H_2O are very similar to each other and to that of **1** reported by *Mehlis et al.* [5]. Negative extrema below 200 nm are characteristic of random-coil peptides [11] [16] [22]. The weak maximum at 223 nm reflects the aromatic contributions of the benzyl groups of Phe-7 and Phe-8 ([5] and *Fig. 1*). A weak minimum around 233 nm is often observed in peptide CD and may be caused by a shoulder of a broad band at lower λ (for a discussion, see [5]). Thus, our spectra are explained by previous work and indicate random coil conformations of [Leu^9]-**1**, **1**, and **2** in H_2O.

The CD of **1** in MeOH is somewhat different from that of *Chassaing et al.* [4], but can be interpreted in a similar manner. The extrema at 196 and 204 nm may indicate a folded structure [4]. However, ellipticity in the 215–240 nm region is significantly less negative than reported [4]. The 224-nm maximum is probably caused by the aromatic amino-acid

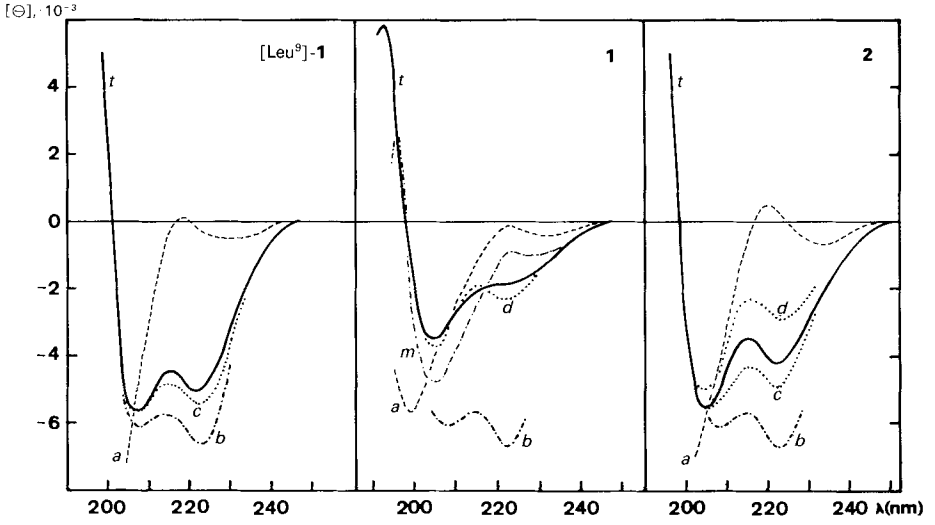

Fig. 3. *CD of [Leu^9]-***1**, **1**, *and* **2** *in H_2O (a), MeOH (m), and CF_3CH_2OH (t) solutions.* [Leu^9]-**1** ($1 \cdot 10^{-4}$ M; *a*, *t*); **1** ($2 \cdot 10^{-4}$ M; *a*, *t*, *m*); **2** ($1 \cdot 10^{-4}$ M; *a*, *t*); *t* was CF_3CH_2OH/H_2O 1:1 (*v/v*). Computed curves taken from [16] are normalized to $[\theta]_r$ at their short wavelength extremum: *b)* 100% α-helix, $[\theta]_r$ (208 nm) = −6200; *c)* 40% random coil, 60% α-helix, $[\theta]_r$ (207 nm) = −5600; *d)* 60% random coil, 40% α-helix, $[\theta]_r$ (205 nm) = −3700 (**1**) and −5000 (**2**).

residues, and the 229-nm minimum may contain contributions of the unidentified peptide shoulder at 233 nm [5]. However, the ellipticities in this region are significantly more negative than in H_2O and do not exclude contributions of α-helices and β-structures proposed by the French authors.

A preliminary study with CF_3CH_2OH as solvent shows that addition of up to 75% (*v/v*) of H_2O to solutions of **1** and **2** in CF_3CH_2OH does not change the spectra significantly. Only at *ca.* 85–90% H_2O, the random-coil spectrum observed in pure H_2O appears. The ordered conformations of the two peptides are, therefore, induced by a relatively small increase in hydrophobicity of the environment.

The negative CD extrema at 205 nm (**1** and **2**) and 208 nm ([Leu^9]-**1**) observed in CF_3CH_2OH and CF_3CH_2OH/H_2O strongly resemble that of **7** in CF_3CH_2OH at 204 nm. Thus, the N-termini of these peptides may have conformations similar to **7**, resembling both poly(proline) [14] and pivaloyl-Pro-Val-NH-Me [15]. The negative ellipticity between *ca.* 215 and 235 nm indicates a significant increase of ordered structures in CF_3CH_2OH and CF_3CH_2OH/H_2O compared with H_2O. Positions and shapes of the CD curves of [Leu^9]-**1**, **1**, and **2** in CF_3CH_2OH are, again, very similar to those of peptides with short helices in $(CF_3)_2CHOH$, *i.e.* HCl·H-(Leu-Leu-Ala)$_n$-OEt and HCl·H-(Met-Met-Leu)$_n$-OEt [22] (see *Table 2*). We assume that the C-terminal segments of **1** are responsible for this resemblance to spectra of helical peptides.

The CD of [Leu^9]-**1**, **1**, and **2** in CF_3CH_2OH and CF_3CH_2OH/H_2O correspond to mixtures of random coil and α-helices without significant β-contributions [16]. Ignoring the influence of the aromatic band, the conformations of [Leu^9]-**1**, **1**, and **2** in CF_3CH_2OH and CF_3CH_2OH/H_2O may be characterized by α-helix/random coil ratios that increase from **1** (40:60) to **2** (50:50) to [Leu^9]-**1** (60:40). These figures are certainly minimal values indicating either the ratio of helix to random coil in every single molecule, or representing an equilibrium condition, or being the result of a combination of the two. The latter seems more probable, although the ratio in [Leu^9]-**1** found here is quite close to that of a molecule comprising a random-coil segment of 4 residues at the N-terminus and an α-helix of 7 residues at the C-terminus (helix/random coil *ca.* 64:36). Moreover, IR amide I band shape analysis [2] indicates 66% α-helix for **1** and **2**, corresponding to 8–9 helical peptide bonds in a total of 13 peptide and carbamoyl groups (**1**), or to 8 in a total of 12 (**2**).

3.4. *Influence of Liposomes on [Leu^9]-***1**, **1**, *and* **2** (see *Fig. 4, Table 2*). The CD of the three peptides in phosphate buffer and in the presence of liposomes prepared from DOPC, from PS, and from PS/DOPC mixtures have been measured. In buffer, the curves are identical to those in H_2O, indicating random-coil structures. Addition of neutral DOPC vesicles has no effect. Hydrophobic interaction of the peptides with neutral liposomes, strong enough to cause a conformational change, is, therefore, excluded. This result agrees with the observations by *Wu et al.* for **1** in solutions of solubilized PC [8] and with those of *Lembeck et al.* [9] on the partitioning of **1** into solutions of PC in MeOH/$CHCl_3$.

With anionic PS liposomes, dramatic changes of the CD are seen. They indicate adsorption of all three peptides to PS liposomes with induction of partially α-helical structures on the membranes. For **1** and [Leu^9]-**1**, the bands at 204 and 207 nm, respectively, are about as intense as the band observed at 205 nm for **1** in solubilized PS [8] (*Table 2*). The extreme at 207 nm of **2** is significantly weaker, perhaps the result of the truncation of the N-terminus (lacking Arg). For [Leu^9]-**1** and **2**, pronounced extrema at

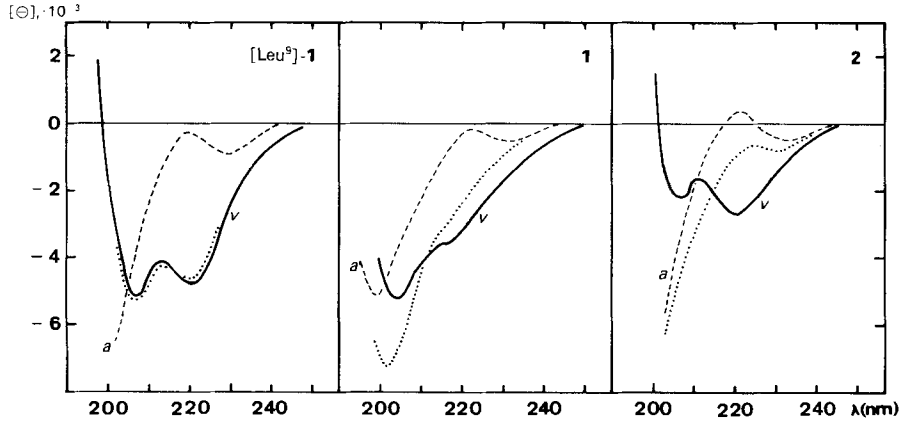

Fig. 4. *CD of [Leu⁹]-***1**, **1**, *and* **2** ($2 \cdot 10^{-4}$ M) *in phosphate buffer* (pH 6.4) *alone and in the presence of liposomes* (12.5 or 15 times by weight). [Leu⁹]-**1**, **1**, and **2** in buffer and with DOPC liposomes (*a*, – – –), in presence of PS liposomes (*v*, ——), and in presence of DOPC liposomes containing 10–20% PS (· · · · ·).

221 nm and their ellipticity ratios *(Table 2)* indicate α-helical structures. For **1**, this band shows up as a shoulder. The intensity of the shoulder is somewhat weaker than that of the 222-nm band of **1** in solubilized PS [8].

The behaviour of the three peptides in the presence of DOPC liposomes containing 10–20% PS is very illuminating. It must be kept in mind that CD 'sees' the peptides both in buffer solution and in contact with vesicles. Thus, changes in the spectra are a measure of the change in the ratio of adsorbed to dissolved peptide. Judging from the CD curves, [Leu⁹]-**1** interacts with PS/DOPC vesicles as strongly as with 100% PS vesicles. The interaction of **1** is significantly reduced, whereas the interaction of **2** is rather weak. This may reflect the expected difference between the hydrophobic interactions of [Leu⁹]-**1** and **1**, caused by the change from Gly-9 to Leu-9 (*ca.* −10 kJ/mol [18]; the net charges of the two peptides are equal, suggesting equal *Boltzmann* distribution near the vesicle surface). The reduced interaction of **2** with PS/DOPC liposomes may reflect its smaller net charge (2+) compared to **1** (3+).

4. Conclusions. – Interpretation of the CD of substance-P peptides is handicapped by the presence of the N-terminal tetrapeptide segment Arg-Pro-Lys-Pro- and the two phenylalanines. The former gives rise to a strong negative extremum at 204 nm that may indicate a conformation of this segment similar to poly(proline) or to pivaloyl-Pro-Val-NHMe in its 'random' state. It influences position and intensity of the helix $\pi \rightarrow \pi^*$ transition extremum between 200 and 210 nm. The phenylalanines give rise to a positive extremum at 217 nm which interferes with the diagnostic 220-nm region, containing contributions from the helix $n \rightarrow \pi^*$ transition. In CF_3CH_2OH solution, the monomeric C-terminal substance-P peptides with 5, 7, and 8 residues adopt helical structures together with mainly random-coil conformers. This agrees with IR studies on **1**, **2**, **4**, and **5** [2]. The proportion of helix is increased in the analogues containing Leu-9 instead of Gly-9. [Leu⁹]-**1**, **1**, and **2** in CF_3CH_2OH show significant amounts of α-helix in combination with random-coil structures, composed mainly of the Arg-Pro-Lys-Pro-segment. This is also the case for these peptides in contact with anionic liposomes prepared from PS. No interaction is observed with neutral DOPC vesicles. With anionic liposomes

prepared from DOPC and 10–20% PS, differences in interaction due to differences in hydrophobicity ([Leu^9]-**1** *vs.* **1**) and in net charge (**1**$^{3+}$ *vs.* **2**$^{2+}$) are readily seen. These results agree with IR and IR-ATR studies [2], and support the predicted membrane structures of **1** and [Leu^9]-**1** [1b] in which N-terminal random coil domains are exposed to the aqueous phase and C-terminal helical domains comprising residues 3–11 interact hydrophobically with the membrane. They also support the predicted accumulation on membranes and helix induction which are, at low peptide concentrations, only possible with anionic membranes due to an electrostatic *Boltzmann* distribution. Participation of residues Lys-3 and Pro-4 in a C-terminal α-helix can only be partial, through their carboxy, but not through their amino groups, and may constitute a transition from the C-terminal α-helix to the N-terminal poly(proline)-like structure.

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