Structure and Optical Activity of Unsaturated Peptides

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Received February 24, 1975

Abstract: α,β-Unsaturated amino acid residues are present in many microbial peptides having antibiotic activity; however, their stereochemistry has not been investigated in detail. In this work 18 N-acylated α,β-unsaturated peptides (dehydropeptides) containing one or two dehydrophenylalanine (dehydro-Phe) residues and a C-terminal optically active L-amino acid or amine residue have been prepared. The structure $CH_3CO[NHC(CHC_6H_5)CO]_nNHC^*HR_1R_2$ (n = 1 and 2) with trans configuration of the double bond in the dehydro-Phe moiety has been demonstrated by NMR, ir, and uv absorption and X-ray diffraction. Monounsaturated peptides show low-intensity CD bands strongly affected by the nature of R_1 and R_2. On the contrary, doubly unsaturated derivatives show CD curves with strong Cotton effects and evidence of exciton splitting with a negative couplet in the 280–240-nm spectral region. The last data are consistent with the existence of an inherently chiral chromophore due to the mutual dissymmetric disposition of the two dehydro-Phe residues. On these bases it is hypothesized that doubly unsaturated peptides have in solution a rigidly fixed conformation probably stabilized by intramolecular hydrogen bonding.

α,β-Unsaturated amino acids have been recently indicated to play some fundamental role in life biological processes. Residues of dehydroalanine are present in the active sites of several enzymes.[2] Recent investigations have shown that a great number of microbial peptides having antibiotic activity are formed from low-molecular-weight peptides containing both α,β-unsaturated and D-amino acid residues. Thus, dehydrovaline is present in penicillin[3] and cephalosporin,[3] dehydroalanine is contained in subtilin[4] and nisin,[5] while both dehydroleucine and dehydrophenylalanine occur in albonoursin.[6]

In fact, a common biosynthetic pathway has been suggested for the formation of dehydro- and D-amino acids. They would not be directly incorporated in the peptides but derived from the L isomer, after incorporation into a biosynthetic intermediate, by a dehydrogenation–hydrogenation sequence.[3] Actually, nonenzymatic hydrogenation of dehydrophenylalanyl derivatives with optically active amino acids gave phenylalanine having both opposite[7] and the same[8,9] absolute configuration with respect to the optically active amino acid originally present in the unsaturated peptide.

A series of unsaturated peptides containing one or more dehydrophenylalanyl (dehydro-Phe) residues was prepared by Bergmann et al.[10] in 1943. At that time detailed investigation of relations between structure and chiroptical properties was not possible due to strong absorption in the near-uv and the lack of information about cis–trans configuration of the double bonds. Indeed, these compounds were expected to exist as a mixture of different stereoisomeric forms.[11]

In the present work the preparation of 18 N-acylated unsaturated peptides containing one or two dehydro-Phe residues has been carried out. These compounds are optically active due to a C-terminal optically active amino acid or amine residue. Relationships between chiroptical properties and primary as well as secondary structure in this series have been investigated with particular reference to the unsaturated moiety.

Results and Discussion

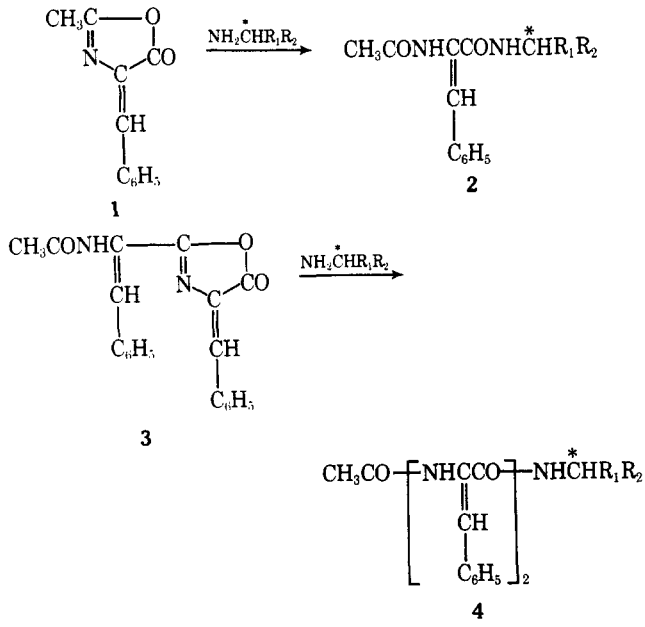
1. Synthesis and Characterization. The reaction sequence previously employed by Bergmann et al.[10] was carried out (Scheme I); thus, the peptides **2a–f,** containing one dehydro-Phe residue, were obtained by reacting the azlactone **1** with the sodium salt respectively of glycine, L-alanine, L-phenylalanine, L-leucine, L-valine, and L-glutamic acid. **2a** was successively treated with benzaldehyde in the presence of acetic anhydride and sodium acetate (Erlenmeyer reaction) to give the unsaturated azlactone **3.** This last was, in turn, used for elongation of the dehydropeptide chain to obtain the doubly unsaturated tripeptides **4.**

All the peptides having a free carboxyl group in the terminal residue (**2a–f, 4a–f**) are soluble in methanol and alkali but are sparingly soluble in the common organic solvents. On the contrary, the methyl ester derivatives are quite soluble in nonpolar solvents like dioxane, chloroform, and dimethoxyethane. These ester derivatives could not be obtained by direct esterification of the carboxyl group with methyl alcohol in the presence of HCl as reported in the literature for analogous compounds.[8] However, they could be obtained in good yields by treating **1** or **3** with an equimolar mixture of amino acid ester–triethylamine in boiling anhydrous chloroform.

NMR spectra at 100 MHz in perdeuterated methanol of all the examined compounds are in agreement with the proposed chemical structure (see Experimental Section). When possible (**2g, 4g, 2h,** and **4h**) the spectra were also recorded in deuteriochloroform; a doublet which can be attributed to the NH proton of the saturated terminal residue is observed in the range 7.64–8.09 ppm. Moreover, in the case of **4g** a peak due to the NH of the unsaturated residues is seen as a sharp singlet downfield (8.50 ppm); on the contrary, in the case of **4h** two separate peaks are present at 8.62 and 8.33 ppm, respectively.

Ir spectra of compounds **2** and **4** in the solid state show, in the region of stretching vibrations of NH and CO groups, a remarkable complexity probably due to intermolecular associations. However, thanks to the large number of compounds examined, bands can be detected which confirm the proposed structure. So around 3150–3250 cm^{-1} bands are present which can be associated with NH of the unsaturated residues, while those of the NH of the optically active residue are centered between 3300 and 3450 cm^{-1}. Moreover, bands are observed of the amide CO between 1600 and 1670 cm^{-1}, of NH bending at 1500–1550 cm^{-1}, and of the monosubstituted benzene ring at 680–780 cm^{-1}. Finally in compounds **2a–g** and **4a–g** the presence of the free car-

Scheme I



	R_1	R_2	
a	H	COOH	glycine
b	CH_3	COOH	L-alanine
c	$CH_2C_6H_5$	COOH	L-phenylalanine
d	$CH_2CH_2CH(CH_3)_2$	COOH	L-leucine
e	$CH_2CH(CH_3)_2$	COOH	L-valine
f	CH_2CH_2COOH	COOH	L-glutamic acid
g	$CH_2C_6H_5$	$COOCH_3$	L-phenylalanine methyl ester
h	CH_3	C_6H_5	L-α-phenylethylamine

boxylic group is consistent with the typical bands at 1750–1700 cm^{-1}.

2. Crystallographic Data. The crystal and molecular structure of *N*-Ac-(dehydro-Phe)$_2$-Gly (**4a**) has been determined. The molecular structure, as viewed along [100], is represented in Figure 1. The bond distances between the heavy atoms are given; the average esd's are 0.005 Å for C–C and 0.004 Å for C–N and C–O bonds.

The two styryl groups are disposed on opposite sides of the peptide chain; both the C(7)–C(8) and C(19)–C(20) double bonds show trans configuration of the carbonyl to the phenyl group; difference arises, however, as regard the conformations around the C(8)–C(9) and C(17)–C(19) bonds, which are s-transoid and s-cisoid, respectively.

The peptide chain contains three complete peptide units and it is interesting to compare their dimensions with those given by Marsh and Donohue,[12] as weighted average of the results of various three-dimensional crystal structure analyses (C^α–C, 1.51 Å; C=O, 1.24 Å; C–N 1.32$_5$ Å; N–C^α, 1.45 Å). The bond distances and valence angles in the peptide unit C(8)C(9)O(11)N(10)C(12) match those given by Marsh and Donohue; on the contrary, the dimensions of the two other peptide units, while matching each other, differ significantly from those of the first one. The lengthening of the N–C bond and the parallel shortening of the C–N bond, relative to the normal values, indicate some conjugation of the nitrogen atom with the lateral styryl group. A complete conjugation would require the coplanarity of the peptide and the styryl groups; the coplanarity is, however, strongly hindered by steric interactions. The strain is differently released in either the two dehydrophenylalanine residues. In fact, one of the residues shows, besides a rotation (42.9°) around the N(16)–C(8) bond, also a rotation (28°) around the C(1)–C(7) bond, with loss of planarity in the styryl

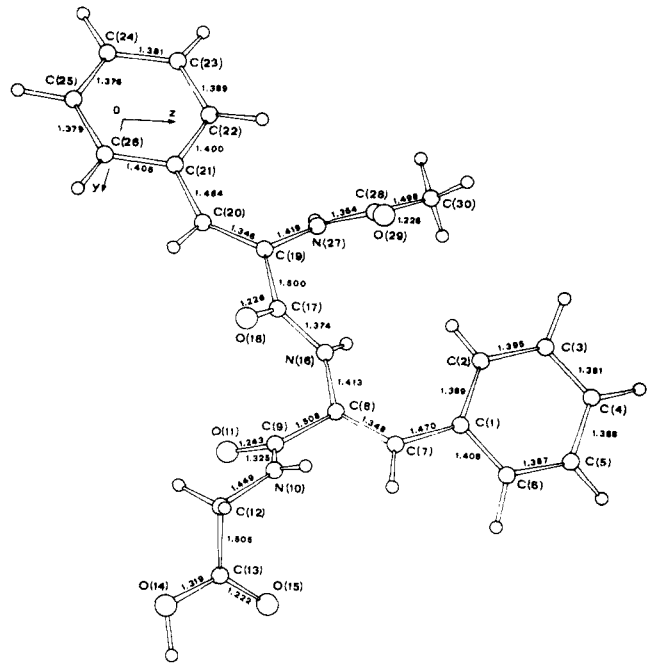


Figure 1. Molecular structure of *N*-Ac-(dehydro-Phe)$_2$-Gly (**4a**).

group itself. The other residue maintains the styryl group relatively planar [with a rotation around C(20)–C(21) of 12.4°] with a large rotation (55°) around C(19)–N(27) bond and a larger value (131.4°) of the valence angle C(19)–C(20)–C(21).

There are also indications of conjugation of the cinnamoyl carbonyl group with its styryl moiety. The groups are as much planar as possible, being the intramolecular contacts O(18)···C(20) and N(10)···C(7) almost at the limit of interatomic contacts. A requirement for conjugation between the above-mentioned unsaturated groups can be hypothesized to explain the retaining of the close contacts.

In the crystal the molecules are held together by hydrogen bonding between carboxyl groups lying across a center of symmetry and, in the [100] direction, by N–H···O hydrogen bonds. The molecules are thus arranged in layers parallel to (001), with a structure analogous to the β-pleated sheet which is observed in polypeptides.

3. Absorption Spectra. Absorption spectra in the near-uv of the dehydropeptides prepared show an intense absorption maximum at 277–279 nm. A shoulder in the longest wavelength side of the curve reveals the existence of a weaker band at about 300 nm. This has been confirmed by obtaining the derivative absorption spectra[13] (Figure 2). These last show a well-defined valley at the wavelength where each shoulder occurs in the absorption spectrum. The mono and doubly unsaturated peptides show different features; particularly, for **2h** (Figure 2, A), where one dehydro-Phe residue is present, only one weak band is apparent in the derivative curve. For **4h** (Figure 2, B) and **4f** (Figure 2, C) which contain two dehydro-Phe residues, two weak bands are apparent at about 295 and 300 nm in the derivative curve.

The position of the 277–279-nm band does not change from compounds **2** to **4**; the molar extinction coefficient ϵ_{max} is 1.8–1.9 × 10^4 for compounds **2** and 3.4–3.8 × 10^4 for compounds **4** (Tables I and II). As in the latter compounds two unsaturated residues per molecule are present; the ϵ_{max} per dehydro-Phe unit is practically constant from mono to doubly unsaturated compounds. As a confirmation of this statement, *N*-Ac-(dehydro-Phe)$_3$-L-Val (**5**) has a value of the ϵ_{max} three times larger than **2** (λ_{max} = 278, ϵ_{max}

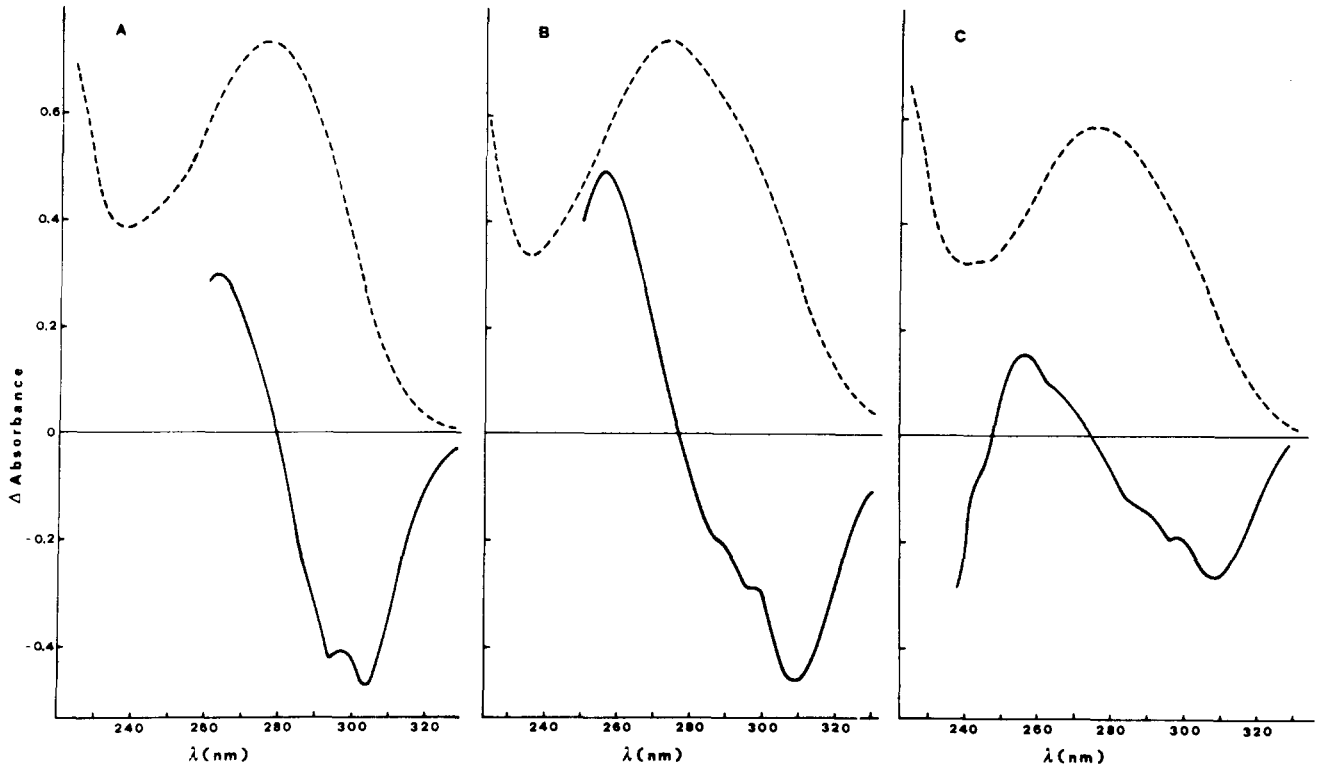


Figure 2. Absorption spectra (- - - -) and first derivative spectra (——) in methanol of A, **2h** (*c* 0.0129 g/l., *l* = 1 cm; derivative curve: Δλ = 4 nm, 0.3 absorption full scale); B, **4h** (*c* 0.0092 g/l., *l* = 1 cm; derivative curve: Δλ = 4 nm, 0.3 absorption full scale); and C, **4f** (*c* 0.0085 g/l., *l* = 1 cm; derivative curve: Δλ = 3 nm, 0.3 absorption full scale).

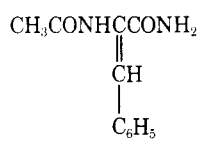
Table I. Optical Properties of Dipeptides Containing One Dehydro-Phe Residue (Methanol Solution)

		Uv absorption		CD data	
Compd[a]	$[\alpha]^{25}D$	λ_{max}, nm	ϵ_{max}	λ_{max}, nm	$[\theta]_{max}$
2b	+65	278	19,000	265	+6,000
2c	−4	279	18,000	290	−3,000
2f	−1	279	18,500	280	−6,000
2g	−37	280	19,000	n.d.	n.d.
2h	+35	279	18,000	280	+15,000
		281[b]	18,000[b]	284[b]	+6,500[b]
8 (*n* = 1)	+109	280	20,000	280	+19,000

[a] The starting optically active reagents had optical purity >99%. [b] In chloroform.

= 5.2 × 10^4). However in the 300–320-nm spectral region a significant increase of the absorption intensity is observed in **4** with respect to **2.**

There is a striking similarity between the absorption spectra of the unsaturated peptides, if referred to one dehydro-Phe residue, and that of *trans*-cinnamic acid.[14] This latter compound shows a strong band at 277 nm with ϵ_{max} 1.8 × 10^4 and a weak band is seen as a shoulder at 296 nm. An analogous absorption spectrum has been reported for α-acetamidocinnamamide (**6**)[15] (λ_{max} = 280 nm; ϵ_{max} = 1.8



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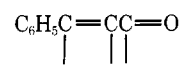

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× 10^4, in methanol). This suggests that the main absorption is due to the same chromophoric system, viz. **7.**

Crystallographic data discussed above for *N*-Ac-(dehydro-Phe)$_2$-Gly (**4a**), as well as those reported in the literature for α-benzamidocinnamates,[16] indicate that the cinnamic moiety **7** is not planar, but rather large skew angles exist between C=C and C=O double bonds. In solution this does not affect appreciably the absorption spectrum since the styrene moiety is mainly responsible for the absorption of the cinnamic chromophore; the effect of the carbonyl group being substantially inductive.[17] If one accepts for **2** the same band assignments as for *trans*-cinnamic acid,[14] the strong absorption band at 277–279 nm can be regarded as an intramolecular charge-transfer band from the highest occupied orbital of the electron-donating styryl group to the vacant orbital of the electron-accepting carbonyl group. The weak band at about 300 nm should correspond to the 260-nm band of benzene.[14]

Similar assignments should be valid for **4.** These last in fact show absorption bands practically at the same wavelength as **2,** whereas the molar extinction is approximately twice (Tables I and II) as large as that of the corresponding bands of monounsaturated peptides and of *trans*-cinnamic acid. This suggests that the dehydro-Phe chromophore is not greatly modified in going from **2** to **4.** The occurrence in the 300-nm region of two bands for **4** and only one for **2** (Figure 2) could be attributed either to the different conformational situation of the two styryl groups or to dipole–dipole interaction.

4. Circular Dichroism Spectra. The CD spectra between 340 and 220 nm of the compounds **4** show marked differences with respect to **2** as far as number, position, and ellipticity of the optically active bands are concerned.

The latter compounds have only low-intensity optically active bands; indeed in some cases it was not possible to detect them due to the very low dissymmetry factor. In general, shape and sign of these bands are strongly affected by the nature of the terminal residue containing the asymmetric carbon atom derived from the optically active amino compounds used in the preparation (see Table I and Figure 3, A).

Table II. Optical Properties of Tripeptides Containing Two Dehydro-Phe Residues (Methanol Solution)

Compd[a]	$[\alpha]^{25}D$	Uv absorption		CD data			
		λ_{max}, nm	ϵ_{max}	λ_{max}, nm	$[\theta]_{max}$	λ_{max}, nm	$[\theta]_{max}$
4b	−178	279	34,000			298	−30,000
4c	−186	277	34,800	275	−42,000	300[b]	−25,000[b]
4d	−200	277	34,000	285	−38,000	295	−38,000
4e	−200	277	34,000	290	−41,000	300[b]	−38,000[b]
4f	−150	279	33,600	285	−25,000	305	−25,000
4g	−168	277	38,000	277	−47,000	300[b]	−27,000[b]
		277[c]	35,000[c]	275[c]	−40,000[c]	310[b,c]	−12,000[b,c]
4h	+12	276	37,000	265	+32,000	305	−5,000
		276[c]	31,000[c]	260[c]	+16,000[c]	300[c]	−12,500[c]
8 (n = 2)	+86	282	37,000	267	+36,000		

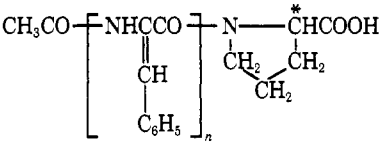
[a] The starting optically active reagents had optical purity >99%. [b] Shoulder. [c] In chloroform.

All compounds **4** having a terminal L-amino acid residue (**4b–f**) show a broad negative dichroic band between 320 and 260 nm which seems to be related to at least two negative Cotton effects and a positive band between 260 and 210 nm (Figure 3, B, and Table II).

This qualitative picture does not change on going to the derivative **4g** where the carboxyl group has been esterified (Figure 4). In this case substitution of polar methanol with chloroform does not produce marked changes in CD spectra at least above 230 nm.

Most interesting is the strict similarity between the curves of compounds **4b–g** independently of type of the amino acid residue present as the chiral end group, but depending on its absolute configuration. This result is a first indication that the optically active chromophore responsible for the shape of CD curves in these compounds and for the dramatic change of chiroptical properties from **2** to **4** must be in the moiety common to all **4b–g** compounds, that is, the system of the two dehydro-Phe residues.

While no substantial differences are observed in CD spectra of **4b–g**, strong variations occur with terminal L-α-phenylethylamine (**4h**) or L-proline (**8**, n = 2) residues,



8 (n = 1 and 2)

where the carboxylic group or the NH group is lacking, respectively, in the end group containing the asymmetric carbon atom.

In the former case (Figure 5) differences in CD spectra of doubly unsaturated (**4h**) and mono-unsaturated (**2h**) derivatives are less marked than in the series **b–g**. The most evident difference is the presence in **4h** of a negative band around 300 nm, which, although of lower ellipticity, corresponds to the analogous band observed in **4b–g**. At shorter wavelengths both **4h** and **2h** show a positive band at 265–275 nm and a negative one centered at about 220 nm. Going from methanol to chloroform the former band ellipticity decreases both in **4h** and **2h**; consequently the negative band present in **4h** at about 300 nm is appreciably increased in intensity.

This last band is not observed in compounds **8** (Figure 6) where no hydrogen atom is present on the nitrogen of the terminal optically active amino acid residue. Again both **8** (n = 1) and **8** (n = 2) have a positive CD band at 280 and 270 nm, respectively, while only in the latter a negative band is observed at 225 nm. The ellipticity of the corresponding bands in the doubly unsaturated derivative is not much higher than that of the monounsaturated compound.

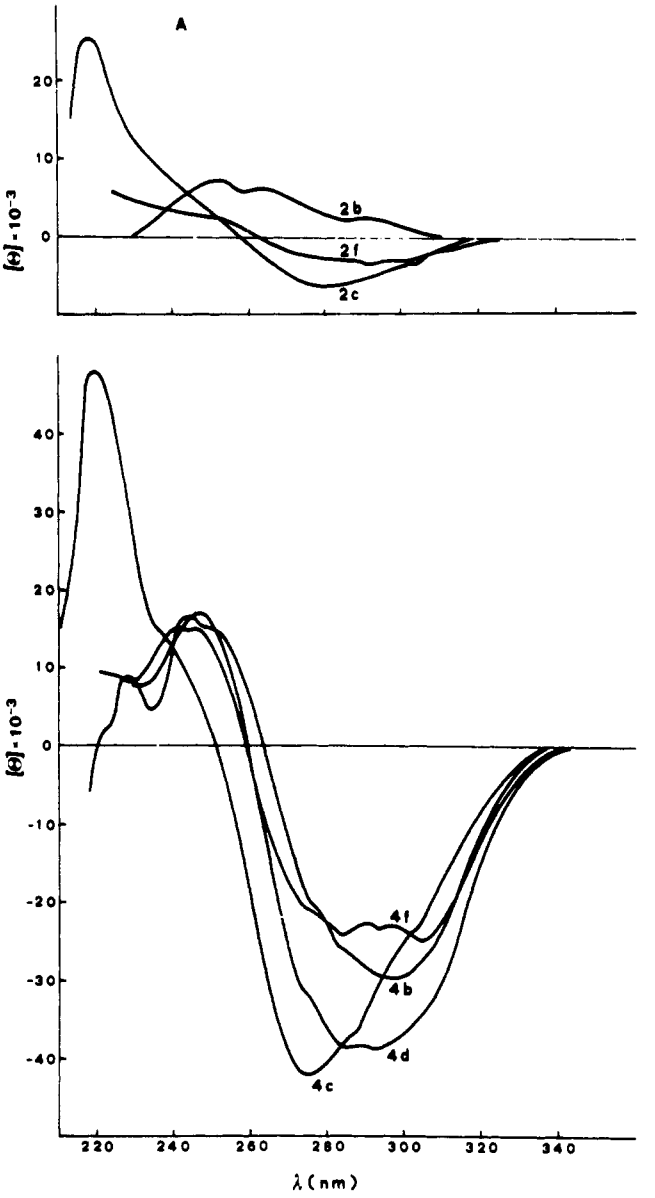


Figure 3. CD spectra, in methanol, of some unsaturated peptides containing one dehydro-Phe residue (A, **2b**, **2c**, and **2f**) or two dehydro-Phe residues (B, **4b**, **4c**, **4d**, and **4f**). **4e** gives practically the same CD curve as **4d**.

In conclusion, the chiroptical properties of compounds **2** can be interpreted on the basis of the dissymmetric perturbation of the dehydro-Phe residue by the asymmetric carbon atom of the terminal amino acid.

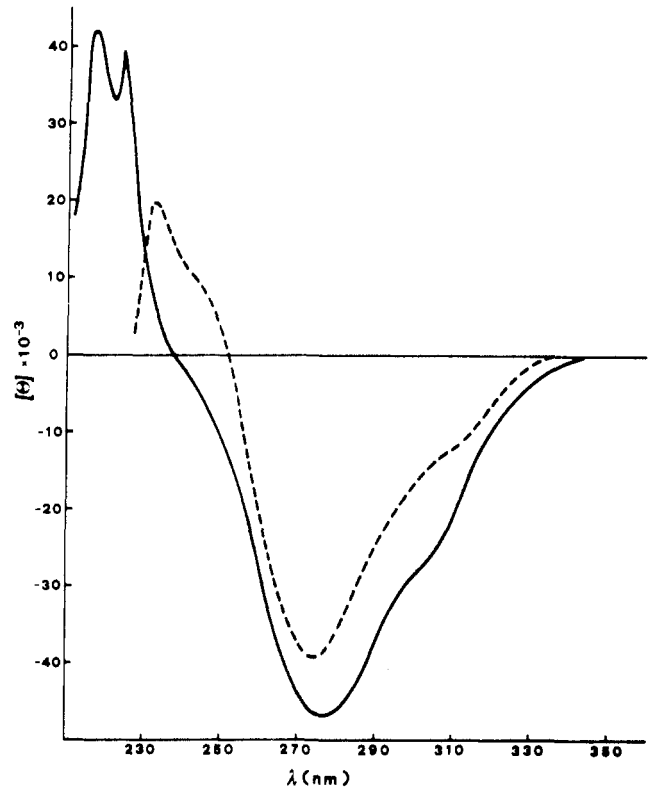


Figure 4. CD spectra of the doubly unsaturated peptide ester **4g** in methanol (——) and in chloroform (- - - -).

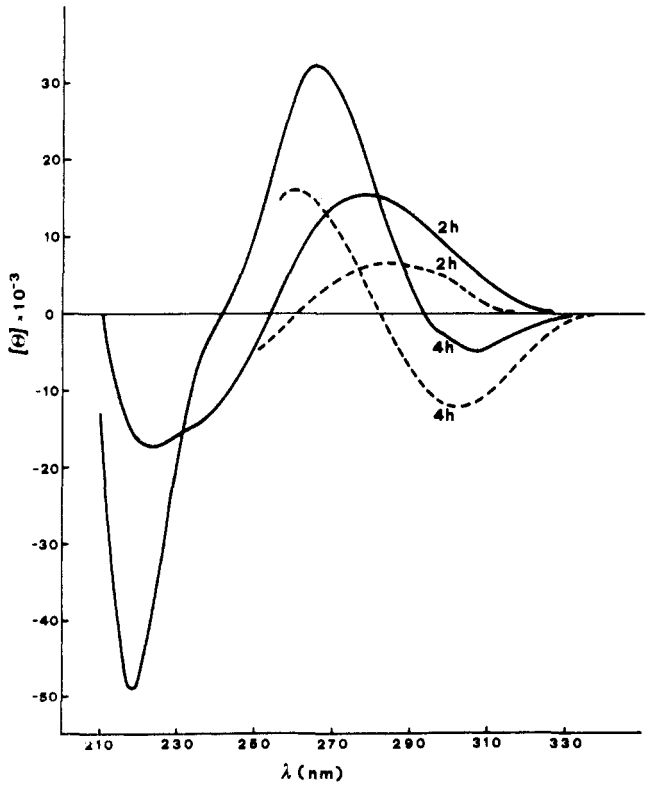


Figure 5. CD spectra in methanol (——) and in chloroform (- - -) of the unsaturated peptides **2h** and **4h** having an L-α-phenylethyl end group.

On the contrary, the magnitude of the Cotton effects and the general features of the CD spectra of **4** are consistent with the presence of an inherently chiral chromophore.

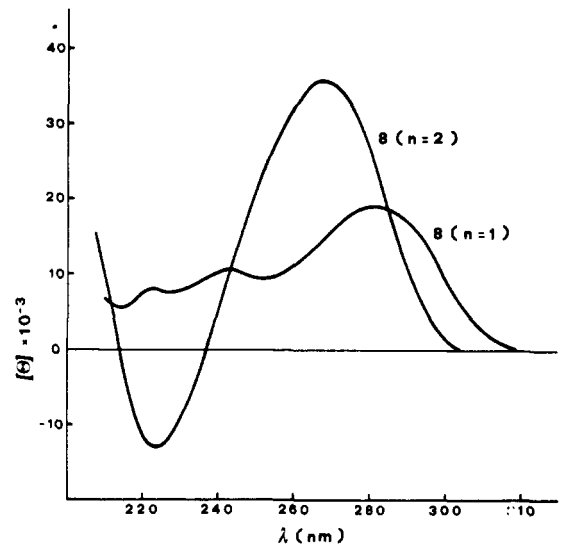


Figure 6. CD spectra, in methanol, of the mono- and the doubly unsaturated peptides **8** containing L-proline as the C-terminal group.

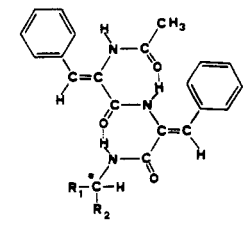
This type of chromophore has been in general observed in cyclic rigidly fixed compounds[18-20] or stereoordered macromolecules where a certain degree of conformation rigidity is provided by cooperative steric interactions between bulky side chains[21,22] and/or by intramolecular hydrogen bonding.[23]

Conformational rigidity of compounds **4** in solution can be expected, because each styryl group tends to be planar as far as possible owing to the conjugation of the unsaturated bonds. This planarity is shown by X-ray data which also demonstrate that the two styryl residues do not lie in the same plane. In fact, steric hindrance forces them to stay in two distinct skewed planes forming, in the crystalline state, a skew angle of approximately 32°.

Because of the symmetric nature of the terminal glycine residue the two skew senses (clockwise and counterclockwise) have the same statistical weight in **4a.** When the terminal amino acid residue is chiral as in **4b–g,** that is, the C(12) of Figure 1 bears an additional substituent, one of the two skew senses should prevail. The effect of this substitution at the C(12) on the skew sense is not evident in the open-chain conformation found in the crystalline state. This conformation, however, is stabilized by intermolecular hydrogen bonds which are not very probable in the diluted methanol or chloroform solution, where a different conformation can be expected. This last conformation is probably stabilized by intramolecular hydrogen bonding.

The "Huggins" type[24] structure **9** (Chart I) proposed for saturated peptides[25] is not very likely in the case of dehydropeptides. In fact, seven-membered ring closure by hydrogen bonding should not occur in this class of compounds due to the unfavorable orientation of the H and O atom, the N-H···O angle being very far from the normal values for hydrogen bonding.[26]

Chart I



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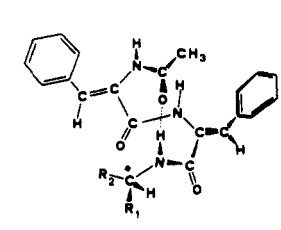

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The correct geometry for hydrogen bonding is obtained only between the N–H group of N(10) of the terminal residue and O(29) of the *N*-acyl end group. This would yield a cyclic structure **10** (Chart I) including the three peptide bonds, with the styryl groups and the group containing the asymmetric carbon atom as side chains.

The importance of the H atom of N(10) in determining the conformation of doubly unsaturated peptides is confirmed by the difference of chiroptical properties between **8**, where this H atom is absent, and compounds **4b–g.** It is of interest to remark that in the structure **10** the asymmetric carbon atom is directly bound to the cycle and therefore it is capable of affecting remarkably its conformation. As a consequence one can expect that also the relative stereochemical position of the two styryl groups is determined by the chiral center.

Transition moments of 277–279-nm absorption bands are directed along the "charge-transfer" line between styryl and carbonyl groups of each dehydro-Phe residue. If these are dissymmetrically disposed it may be expected that the coulombic coupling of these excitation moments give rise to exciton splitting,[27] thus producing the couplet between 240 and 280 nm. Clearly, coupling between other transitions can also occur, but the complexity of the CD spectra does not allow at present to answer this question.

Around 300 nm, where the short-axis polarized electronic transition of the styryl chromophore is located, no CD band or only an extremely weak band is observed in the case of **2**, whereas a strong negative band is present in the case of **4b–g.** This increase of ellipticity should be attributed to the partially hindered rotation of the aromatic groups[19,28] according to crystallographic data.

Conclusions

The determination of structure of the wide series of dehydrophenylalanyl peptides allows us to conclude that the preparation method of unsaturated peptides by reaction of azlactones with amino acids salts originally proposed by Bergmann et al.[10] is stereospecific and gives double bonds with trans configuration both in mono (**2**) and doubly unsaturated (**4**) derivatives.

Crystallographic data provide evidence for a certain degree of rigidity in the dehydro-Phe chromophoric system; in the case of doubly unsaturated peptides **4** steric hindrance forces the two unsaturated groups to stay in two distinct skewed planes.

Absorption in the near-uv of both **2** and **4** is determined substantially by the dehydro-Phe chromophore, the latter compounds showing the same pattern as the former, with double intensity.

Large differences between the two series **2** and **4** are observed in CD spectra in the same spectral region.

Compounds **2** possess low-intensity bands, strongly affected by the structure of the optically active end group. They can be explained on the basis of the asymmetric perturbation of the dehydro-Phe chromophore and do not suggest any particular rigid conformation such as the Huggins type structure.

On the contrary, all the compounds **4b–g** show very similar patterns with strong Cotton effects associated with a inherently chiral chromophore. This last originates from the dissymmetric disposition of the two unsaturated residues in two skewed planes, in a rigid cyclic conformation stabilized by intramolecular hydrogen bonding.

The complex structure of the molecules examined does not permit at present the determination of the predominant skew sense when C* has L absolute configuration. However, a definite relation is demonstrated by the close similarity of the CD spectra of **4b–g,** an L asymmetric carbon atom giving a negative band around 300 nm and a negative couplet between 280 and 240 nm. X-Ray analysis of the optically active derivatives containing two dehydro-Phe residues seems to be necessary before establishing a relation between sign of the couplet and chirality of the molecule.

Experimental Section

(a) Methods. The NMR spectra were obtained on a Varian HA-100-15 high-resolution spectrometer; chemical shifts are reported in δ (parts per million) with tetramethylsilane as the internal reference.

Optical measurements were carried out on solutions in Merck's Uvasol solvents.

The uv absorption spectra were obtained with an SP 700 Unicam spectrophotometer. The derivative spectra were recorded with the Hitachi Perkin-Elmer Model 356 two-wavelength double beam spectrophotometer. The curves were obtained with wavelength differences $\Delta\lambda$ = 2, 3, and 4 nm and expanded scale ranges of absorbance, 0–0.1, 0–0.3, full scale.

Optical rotations at the sodium D line were measured with a Perkin-Elmer Model 141 polarimeter.

CD curves were recorded at 27° using a Jouan-Roussel dichrograph 185 II and a Cary Model 60 spectropolarimeter equipped with a CD Model 6001 accessory. The molar ellipticity $[\theta]$ is given as deg cm^2 dmol^{-1}.

Ir spectra were obtained by a Perkin-Elmer Model 225 spectrophotometer.

Crystal Data and Molecular Structure Determination of *N*-Ac-(dehydro-Phe)$_2$-Gly (4a). The compound crystallizes in space group $P\bar{1}$, with cell constants: a = 8.75, b = 11.79, c = 10.44 Å; α = 103° 41′, β = 98° 24′, γ = 100° 49′. A total of 2860 independent structure amplitudes was obtained from microdensitometer measurements on integrated Weissenberg photographs taken with Cu Kα radiation. The structure was solved by means of the symbolic addition method and refined by the least-squares method to a reliability index 0.072 for all the observed reflexions. The hydrogen atoms were located by means of a difference synthesis.

(b) Materials. Some typical preparations are reported.

Ac-(dehydro-Phe)$_2$-L-Ala (4b) was obtained by treatment of the azlactone **3** with L-alanine according to the procedure of Bergmann et al.[10] Analytical data: mp 207–208°; ir (Nujol) ν_{NH} 3395 (m) 3230 (sh), 3160 cm^{-1} (sh); ν_{COOH} 1772 cm^{-1} (s); ν_{CO} 1660 (s), 1642 (s), 1632 cm^{-1} (s); NMR (CD_3OD) 1.51 (d, 3, CH_3C*), 2.12 (s, 3, CH_3CO), 4.56 (m, 1, CH), 7.05 (s, 2, CH=C), 7.39–7.49 (m, aromatic). Anal. Calcd: C, 65.50; H, 5.20; N, 10.35. Found: C, 65.75; H, 5.30; N, 10.05.

Ac-(dehydro-Phe)$_2$-L-Glu (4f). It was obtained as described by Bergmann et al.[10] and had the following analytical data: mp 193–194°; ir (Nujol) ν_{NH} 3355 (s), 3225 (s), and 3170 cm^{-1} (sh); ν_{COOH} 1740 (s) and 1705 cm^{-1} (s); ν_{CONH} 1660 (s), 1630 (s), and 1620 cm^{-1} (s); NMR (CD_3OD) 2.14 (s, 3, CH_3CO), 2.44 (m, 4, $-CH_2CH_2-$), 4.56 (m, 1, CH), 7.06 (s, 2, 2CH=C), 7.35–7.49 (m, aromatic).

Ac-(dehydro-Phe)$_2$-L-Phe Me Ester (4g). To a solution of 1.0 g (3 mmol) of the azlactone **3** in 20 ml of dry chloroform was added 5 ml of a solution containing 0.64 g (3 mmol) of phenylalanine methyl ester hydrochloride and 0.30 g (3 mmol) of NEt_3. The reaction mixture was allowed to reflux for 8 hr; then the solvent was removed under reduced pressure. The residue was washed with water and recrystallized from methanol–water and chloroform–petroleum ether: mp 188–189°; ir (Nujol) ν_{NH} 3415 (m), 3345 (m), and 3330 cm^{-1} (m); ν_{COOR} 1744 cm^{-1} (s); ν_{CONH} 1680 (s), 1660 (s), 1637 (s), and 1626 cm^{-1} (s); NMR ($CDCl_3$) 2.02 (s, 3, CH_3C=O), 3.06 (d, 2, CH_2), 3.50 (s, 3, OCH_3), 4.64 (m, 1, *CH), 6.80, 7.02 (2 s, 1, 2CH=C), 7.13–7.28 (m, aromatic), 7.64 (d, 1, NHC*), 8.47 (s, 2, 2NHC=C). Anal. Calcd: C, 70.40; H, 5.68; N, 8.22. Found: C, 70.55; H, 5.70; N, 8.08.

Ac-(dehydro-Phe)$_2$-L-α-phenylethylamide (4h). The azlactone **3** (13.8 g, 42 mmol) was dissolved in 100 ml of dry benzene containing 5.1 g (42 mmol) of L-α-phenylethylamine ($[\alpha]^{25}D$ −40.2) and the reaction mixture refluxed for 2 hr. Then the mixture was poured into an excess of 0.5 *N* HCl and the filtered product recrystallized from methanol–water: mp 201–204°; ir (Nujol) ν_{NH} 3295 (m), 3220 (m) and 3150 cm^{-1} (sh); ν_{CONH} 1674 (m), 1646 (s), and 1625 cm^{-1} (s); NMR ($CDCl_3$) 1.41 (d, 3, CH_3C*), 1.95 (s, 3,

$CH_3C{=}O$), 4.78 (m, 1, *CH), 6.58 (s, 2, 2CH=C), 7.06-7.28 (m, aromatic), 8.09 (d, 1, NHC*), 8.33, 8.62 (2 s, 1, 2NHC=C).

Anal. Calcd: C, 74.15; H, 6.01; N, 9.26. Found: C, 74.90; H, 6.06; N, 9.00.

Acknowledgment. The authors wish to express their thanks to Mr. P. Vergamini for recording ir spectra, to Mr. C. Bertucci for CD spectra, and Professor P. A. Temussi for NMR.

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Use of Carbon-13 Nuclear Magnetic Resonance to Establish That the Biosynthesis of Tropic Acid Involves an Intramolecular Rearrangement of Phenylalanine

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Abstract: The administration of DL-[1-^{14}C,1,3-^{13}C]phenylalanine (containing 81% of the $^{13}C_2$ species) to *Datura innoxia* plants yielded labeled hyoscyamine and scopolamine. Proton noise decoupled ^{13}C NMR spectra of these enriched alkaloids revealed the presence of satellite peaks, due to ^{13}C-^{13}C spin-spin coupling, symmetrically located about the singlet peaks arising from C-1 and C-2 of the tropic acid moiety of these alkaloids. This result indicates that the rearrangement of phenylalanine to tropic acid involves an *intra*molecular migration of the carboxyl group. Hyoscyamine, scopolamine, and phenylalanine isolated from *Datura stramonium* plants which had been fed [1-^{14}C]phenylacetic acid had negligible activity, indicating that phenylacetic acid is not involved in the biosynthesis of tropic acid.

Tropic acid (**2**) is found in Nature as the acid moiety of the ester alkaloids hyoscyamine (**3**) and scopolamine (**4**). In 1960 it was discovered[2] that the administration of [3-^{14}C]phenylalanine (**1**) to intact *Datura stramonium* plants yielded labeled tropic acid having essentially all its activity located at C-2. Later workers confirmed this result in *D. stramonium* (intact plants)[3] and *D. metel* (root cultures).[4] It was then established that the other carbons of the phenylalanine side chain were utilized for the formation of tropic acid.[5,6] The tropic acid formed from [1,3-^{14}C]phenylalanine had the same ratio of activity at C-1 to C-2 as C-1 to C-3 in the administered phenylalanine.[7,8] These results suggested that a molecular rearrangement of the side chain of phenylalanine was occurring. Despite extensive subsequent tracer work[9,10] the mechanism of this rearrangement is unknown. This reaction may be related to the conversion of succinyl coenzyme A to methylmalonyl coenzyme A which is catalyzed by a transcarboxylase from propionic acid bacteria.[11]

It has been reported[3,12] that phenylacetic acid is a precursor of tropic acid. The activities of degradation products of tropic acid derived from [1-^{14}C]phenylacetic acid were consistent with specific labeling of the tropic acid at C-3. It was suggested[3] that phenylpyruvic acid (**5**) formed from phenylalanine by transamination undergoes an oxidative decarboxylation yielding phenylacetyl coenzyme A (**7**) and carbon dioxide (possibly bound to a coenzyme such as biotin). Carboxylation of **7** then affords phenylmalonyl coenzyme A (**6**) which on reduction yields tropic acid, as illustrated in Scheme I. Another plausible route to tropic acid is shown in Scheme II. It has been established that polyporic acid (**8**) and related 2,5-diphenylbenzoquinones are formed by the condensation of two molecules of phenylpyruvic acid derived from phenylalanine.[13] A cleavage of the polyporic acid (indicated by a dotted line) would result in the formation of two C_6-C_3 units having the skeleton of tropic acid and a distribution of the side-chain carbon atoms consistent with the previously described tracer experiments.