

α,β -Dehydro Residues in the Design of Peptide and Protein Structures

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Abstract

The results of X-ray studies on 19 structures containing dehydro residues have been analysed. The observed average $C^\alpha=C^\beta$ distance in the dehydro residues is 1.331 (2) Å. The average values of the $C^\alpha=C^\beta-C^\gamma$ bond angle in dehydro-Phe and dehydro-Leu are 131.2 (2) and 127.3 (1)°, respectively. The dehydro residue is essentially planar. A β -turn of type II is formed if the dehydro residue is placed either at the ($i+1$) or at the ($i+2$) corner position of the β -turn. If the dehydro residues occur consecutively in an amino-acid sequence, the backbone folds into an alternating right- and left-handed α -helix. The peptide bond is planar in all these structures. The β -turn is stabilized by an intramolecular hydrogen bond between CO of the i th and NH of the ($i+3$)th residue.

Introduction

α,β -Unsaturated (or dehydro) amino acids have been found to occur naturally in antibiotics of microbial origin (Gross & Morell, 1967, 1968; Gross, Morell & Craig, 1969; Aydin, Lucht, Koenig, Lupp, Jung & Winkelmann, 1985; Allagier, Jung, Werner, Schneider & Zaehner, 1986) and in some proteins, e.g. histidine ammonia lyase from bacterial and mammalian sources and phenylalanine ammonia lyase from plants (Noda, Shimohigashi & Izumiya, 1983). Peptides containing α,β -dehydro residues are synthesized in the ribosome *via* a precursor protein followed by enzymatic modification (Allagier, Jung, Werner, Schneider & Zaehner, 1986). Furthermore, several active analogues of naturally occurring peptides have been synthesized with dehydro amino-acid substitution. The first synthesis of an α,β -dehydro amino acid was carried out by Ploechl (1883). The recent vigorous revival of interest in this area is a result of the discovery of many dehydro amino acids in nature. Moreover, incorporation of dehydro amino-acid residues into peptide antibiotics, peptide hormones and enzymes has provided analogues exhibiting modified bioactivity. These have been useful for establishing structure–activity relationships. The introduction of a dehydro residue (Fig. 1) into an amino-acid sequence may lead to specific secondary

peptide structures (Singh & Chauhan, 1988). The majority of such structures have been designed with either dehydro-Phe or dehydro-Leu at the corner positions $i+1$ or $i+2$ of the β -turn (Fig. 2). In this paper we compare the structures of 19 peptides which contain dehydro residues (Δ^2).

Experimental background

So far, the structures of 19 peptides containing dehydro residues have been determined by X-ray diffraction (Table 1). These peptides will be referred to hereafter as *A, B, C... etc.* as given in Table 1. The values of the bond lengths and angles of dehydro residues in various peptides are listed in Tables 2–6. The weighted-average values of the bond lengths and angles calculated using the formula given by Topping (1961) are listed in the last row of these tables. The average values will be used in the subsequent discussion.

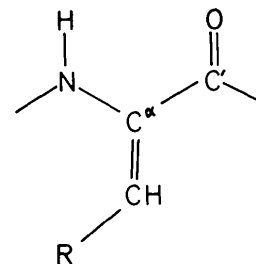


Fig. 1. Dehydro amino-acid residue.

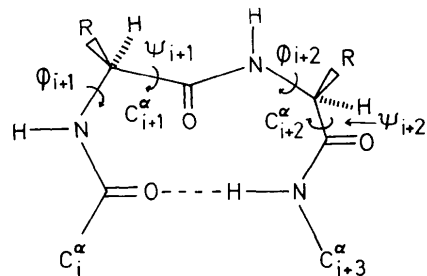


Fig. 2. A β -turn.

Table 1. Structures of peptides containing dehydro residues determined by X-ray diffraction

(i) Dehydro-Phe-containing peptides		
A	N-Ac-dehydro-Phe-OH	Ajo, Casarin, Granozzi & Busetti (1981)
B	N-Ac-dehydro-Phe-OCH ₂ CH ₃	Ajo, Busetti, Ottenheim & Plate (1984)
C	N-Ac-dehydro-Phe-NHCH ₃	Aubry, Allier, Boussard & Marraud (1985)
D	N-Ac-dehydro-Phe-L-Pro-OH	Ajo, Busetti & Granozzi (1982)
E	N-Boc-L-Gly-dehydro-Phe-NHCH ₃	Singh, Narula, Chauhan & Kaur (1989)
F	N-Phenylmethoxycarbonyl-L-Phe-dehydro-Phe-OH	Głowska, Gilli, Bertolasi & Makowski (1987)
G	N-Boc-L-Pro-dehydro-Phe-L-Gly-OH	Patel, Singh, Chauhan & Kaur (1990)
H	N-Bu-CO-L-Pro-dehydro-Phe-NHCH(CH ₃) ₂	Aubry, Boussard & Marraud (1984)
I	N-Boc-L-Phe-dehydro-Phe-L-Val-OCH ₃	Singh, Haridas, Chauhan & Kumar (1987)
J	N-Boc-L-Gly-dehydro-Phe-L-Gly-OCH ₃	Głowska (1988)
K	N-(Carbobenzoyloxy)-L-Phe-dehydro-Phe-L-Gly-OCH ₃	Galdecki (1986)
L	N-Ac-dehydro-Phe-dehydro-Phe-L-Gly-OH	Pieroni, Montagnoli, Fissi, Merlino & Ciardelli (1975)
M	N-Ac-dehydro-Phe-dehydro-Phe-L-Ala-OH	Pieroni, Fissi, Merlino & Ciardelli (1976/1977)
(ii) Dehydro-Leu-containing peptides		
N	N-Boc-dehydro-Leu-OH	Chauhan, Stammer, Norskov-Lauritzen & Newton (1979)
O	N-Boc-L-Pro-dehydro-Leu-OCH ₃	Narula, Patel, Singh, Chauhan & Sharma (1988)
P	N-Boc-L-Pro-dehydro-Leu-NHCH ₃	Singh, Narula, Chauhan, Sharma & Hinrichs (1989)
Q	N-Boc-L-Phe-dehydro-Leu-L-Val-OCH ₃	Narula, Patel, Singh & Chauhan (1990)
(iii) Dehydro-Ala sequence		
R	N-Ac-dehydro-Ala-OH	Ajo, Granozzi, Tondello, Del Pra & Zanotti (1979)
(iv) Dehydro-Pro sequence		
S	N-Ac-dehydro-Pro-OH	Ajo, Busetti, Granozzi & Liakopoulou-Kyriakides (1984)

Table 2. Bond lengths (Å) of the dehydro-Phe moiety in various peptides

The coordinates of structures *H* and *K* are not available and the bond lengths are not reported by the authors.

Peptide	N—C ^α	C ^α —C ^γ	C ^α =O	C ^α =C ^β	C ^β —C ^γ
A	1.438 (9)	1.496 (9)	1.212 (7)	1.319 (9)	1.450 (9)
B*	I 1.412 (9)	1.467 (8)	1.199 (7)	1.327 (11)	1.455 (10)
	II 1.410 (7)	1.477 (10)	1.211 (7)	1.329 (10)	1.472 (8)
C	1.414 (3)	1.514 (6)	1.234 (8)	1.324 (6)	1.493 (8)
D	1.425 (7)	1.519 (7)	1.247 (6)	1.324 (8)	1.493 (8)
E	1.425 (6)	1.513 (7)	1.246 (6)	1.324 (6)	1.448 (7)
F	1.421 (2)	1.488 (3)	1.210 (3)	1.327 (3)	1.465 (3)
G	1.42 (2)	1.49 (2)	1.29 (2)	1.33 (2)	1.45 (2)
I	1.394 (6)	1.522 (7)	1.217 (6)	1.348 (8)	1.462 (8)
J	1.427 (2)	1.489 (3)	1.237 (2)	1.334 (3)	1.464 (3)
L†	I 1.419 (3)	1.500 (3)	1.226 (3)	1.348 (3)	1.464 (4)
	II 1.413 (3)	1.505 (3)	1.243 (3)	1.348 (3)	1.470 (4)
M†	I 1.423 (2)	1.513 (3)	1.239 (3)	1.312 (3)	1.469 (4)
	II 1.422 (3)	1.525 (3)	1.231 (3)	1.319 (3)	1.471 (3)
Weighted-average values	1.421 (2)	1.504 (2)	1.231 (2)	1.331 (2)	1.462 (2)

* Two molecules in the asymmetric unit.

† I, dehydro-Phe at the (*i* + 1) position; II, dehydro-Phe at the (*i* + 2) position.

Bond lengths

In all these structures the average C^α=C^β double-bond length is 1.331 (2) Å. The N—C^α distance is 1.421 (2) Å whereas the corresponding average length in saturated residues is 1.45 Å (Benedetti, 1977). The length of the C^α—C^γ bond is 1.504 (2) Å

Table 3. Bond angles (°) of the dehydro-Phe moiety in various peptides

The coordinates of structures *H* and *K* are not available and the bond angles are not reported by the authors.

Peptide	C—		N—		C ^α —	
	N—C ^α	C ^α —C ^γ	C ^α —C ^β	C ^α —C ^β	C ^α —C ^γ	C ^β —C ^γ
A	121.7 (5)	115.5 (4)	125.1 (5)	119.4 (4)	132.1 (5)	132.6 (5)
B*	I 123.2 (5)	115.1 (5)	122.2 (6)	122.7 (6)	132.6 (6)	132.6 (6)
	II 120.0 (5)	114.9 (5)	121.7 (5)	123.8 (5)	127.0 (5)	127.1 (4)
C	122.2 (4)	114.6 (4)	123.5 (4)	121.3 (4)	127.1 (4)	129.3 (6)
D	120.0 (5)	113.4 (5)	126.2 (5)	120.2 (5)	129.3 (6)	129.3 (6)
E	121.5 (4)	116.7 (4)	124.6 (4)	118.7 (4)	133.6 (5)	133.6 (5)
F	123.0 (2)	117.6 (1)	124.7 (2)	117.6 (2)	131.8 (2)	131.8 (2)
G	120 (1)	116 (1)	124 (1)	120 (1)	133 (1)	133 (1)
I	121.6 (3)	118.3 (4)	126.9 (5)	114.1 (5)	132.0 (5)	132.0 (5)
J	121.1 (1)	116.7 (2)	124.3 (2)	118.9 (2)	132.2 (2)	132.2 (2)
L†	I 123.3 (2)	115.0 (2)	127.7 (2)	117.1 (2)	133.2 (2)	133.2 (2)
	II 121.9 (2)	113.5 (2)	125.8 (2)	120.4 (2)	128.5 (2)	128.5 (2)
Weighted-average values	121.2 (1)	116.3 (1)	125.3 (2)	118.9 (2)	131.1 (2)	131.1 (2)

* Two crystallographically independent molecules in the asymmetric unit.

† I, dehydro-Phe at the (*i* + 1) position; II, dehydro-Phe at the (*i* + 2) position.

Table 4. Bond lengths (Å) of the dehydro-Leu moiety in various peptides

Peptide	N—C ^α	C ^α —C ^γ	C ^α =O	C ^α =C ^β	C ^β —C ^γ
N*	I 1.421 (2)	1.497 (3)	1.254 (1)	1.323 (1)	1.496 (3)
	II 1.420 (2)	1.488 (3)	1.263 (1)	1.323 (1)	1.497 (3)
O*	I 1.41 (1)	1.47 (2)	1.18 (2)	1.33 (2)	1.46 (2)
	II 1.41 (1)	1.48 (2)	1.20 (1)	1.31 (2)	1.44 (2)
P	1.45 (2)	1.46 (2)	1.25 (2)	1.33 (2)	1.49 (3)
Q	1.430 (7)	1.474 (10)	1.246 (8)	1.323 (9)	1.496 (13)
Weighted-average values	1.421 (2)	1.491 (2)	1.258 (2)	1.323 (2)	1.468 (2)

* Two crystallographically independent molecules in the asymmetric unit.

Table 5. Bond angles (°) in the dehydro-Leu moiety in various peptides

Peptide	C—		N—		C ^α —	
	N—C ^α	C ^α —C ^γ	C ^α =C ^β	C ^α =C ^β	C ^α —C ^γ	C ^β —C ^γ
N*	I 125.1 (1)	116.8 (1)	123.0 (1)	119.7 (1)	127.1 (1)	127.1 (1)
	II 125.1 (1)	117.0 (1)	122.8 (1)	119.6 (1)	127.4 (1)	127.4 (1)
O*	I 121 (1)	115 (1)	123 (1)	122 (1)	128 (1)	128 (1)
	II 122 (1)	115 (1)	122 (1)	121 (1)	129 (1)	129 (1)
P	123 (1)	117 (1)	120 (1)	124 (1)	126 (1)	126 (1)
Q	121.8 (6)	117.3 (6)	122.0 (7)	120.2 (7)	129.4 (8)	129.4 (8)
Weighted-average values	123.2 (2)	116.9 (1)	122.9 (1)	119.9 (6)	127.3 (1)	127.3 (1)

* Two crystallographically independent molecules in the asymmetric unit.

while in saturated residues this distance is 1.53 Å (Benedetti, 1977). The weighted-average value of the C^γ=O bond is 1.231 (2) Å in dehydro-Phe and 1.258 (2) Å in dehydro-Leu residues. These distances are slightly larger than those found in saturated Phe and Leu residues (Benedetti, 1977). The average C^β—C^γ distance is 1.462 (2) Å. The systematic shortening of the N—C^α, C^α—C^γ and C^β—C^γ single bonds, and the elongation of the C^γ=O double bond in dehydro residues can be explained by an increased planarity caused by the C^α=C^β double bond which

Table 6. Bond lengths (Å), bond angles (°) and torsion angles (°) of dehydro-Ala and dehydro-Pro moieties

	N—C ^α	C ^α —C'	C'=O	C ^α =C ^β	C ^β —C ^γ	C ^γ —C ^δ	C ^δ —N	
R	1.409 (5)	1.504 (4)	1.210 (5)	1.328 (5)				
S	1.416 (5)	1.485 (6)	1.248 (5)	1.313 (6)	1.490 (6)	1.517 (7)	1.478 (6)	
	C—N—C ^α	N—C ^α —C'	N—C ^α =C ^β	C'—C ^α =C ^β	C ^α =C ^β —C ^γ	C ^β —C ^γ —C ^δ	C ^γ —C ^δ —N	C ^δ —N—C ^α
R	126.8 (4)	110.8 (3)	127.9 (3)	121.3 (4)	—	—	—	—
S	124.8 (3)	121.9 (4)	110.7 (4)	127.2 (4)	111.9 (4)	103.6 (4)	104.6 (4)	108.7 (3)
	φ	χ ¹	χ ²	χ ³	χ ⁴	θ ^o		
R	179.2 (3)	—	—	—	—	—		
S	-26.3 (7)	0.9 (6)	3.8 (6)	-6.6 (5)	7.5 (5)	-5.5 (5)		

results in extended delocalization of the π -electron system.

Bond angles

The bond angles involving non-hydrogen atoms of dehydro residues are given in Tables 2–6. The value of the N—C^α—C' bond angle is less than the standard value 120° while those of N—C^α=C^β and C^α=C^β—C^γ are substantially larger. The closing of the former bond angle and the simultaneous opening of the latter two bond angles suggest a mechanism in which the steric constraints arising from the dehydro residues are released. The values of the C^α=C^β—C^γ angle in dehydro-Phe and dehydro-Leu residues suggest that the effects of steric constraints in dehydro-Phe are far more pronounced than those in dehydro-Leu. In other words, the degree of flatness of the residue and the size of the side chain are two important parameters determining the geometrical features of dehydro residues.

Discussion

β -turns were first recognized by Venkatachalam (1968). These turns are responsible for chain reversal in polypeptide structures (Smith & Pease, 1980; Birktoft & Blow, 1982). These structures were originally classified into three major categories: type I, $\varphi_{i+1} = -60$, $\psi_{i+1} = -30$, $\varphi_{i+2} = -80$, $\psi_{i+2} = 0^\circ$; type II, $\varphi_{i+1} = -60$, $\psi_{i+1} = 120$, $\varphi_{i+2} = 80$, $\psi_{i+2} = 0^\circ$; type III, $\varphi_{i+1} = -60$, $\psi_{i+1} = -30$, $\varphi_{i+2} = -60$, $\psi_{i+2} = -30^\circ$; and the corresponding enantiomeric structures I', II' and III', where the signs of all the φ and ψ angles are reversed. In all these structures a hydrogen bond between CO of residue i and NH of residue $i+3$ stabilizes the folded conformation.

Conformation of the backbone with a dehydro-Phe residue

The torsion angles φ and ψ in the dehydro-Phe residue when dehydro-Phe is placed in different sequences and at different positions in the β -turn are given in Table 7. As seen from Table 7 (E–J), the values of φ and ψ are in the vicinity of 80 and 0°, respectively, if the dehydro-Phe residue is placed at

Table 7. Torsion angles (°) of the dehydro-Phe moiety in various peptides

		φ	ψ	χ ¹	χ ^{2,1}	χ ^{2,2}
(i) Dehydro-Phe alone						
A		71.7 (9)	—	0.8 (13)	-1.3 (13)	171.9 (8)
B*	I	80.8 (7)	—	0.0 (1)	-18.0 (1)	165.7 (8)
	II	-64.0 (8)	—	-5.0 (1)	-34.9 (10)	150.2 (7)
(ii) Dehydro-Phe at the (i + 1) position						
C		-58.3 (4)	148.0 (5)	-7.8 (4)	-37.5 (4)	143.3 (4)
D		-51.5 (11)	135.2 (8)	-5.1 (15)	35.4 (15)	-140.5 (10)
(iii) Dehydro-Phe at the (i + 2) position						
E		71.5 (6)	7.2 (6)	1.6 (9)	0.5 (9)	179.8 (9)
F		50.4 (3)	—	2.7 (4)	16.0 (4)	-164.2 (2)
G		65 (2)	15 (2)	-1 (2)	8 (2)	-176 (1)
H		63	10	-4	-3	169
I		46.5 (2)	38.0 (4)	0.0 (3)	28.8 (3)	-156.9 (2)
J		-69.3 (2)	-11.4 (2)	2.1 (3)	2.2 (3)	-177.3 (3)
K†						
(iv) Dehydro-Phe at both the (i + 1) and (i + 2) positions						
L‡	I	42.9 (3)	51.2 (4)			
	II	-55.2 (3)	-49.6 (4)			
M‡	I	41.0 (3)	55.3 (3)	3.3 (4)	6.8 (4)	-174.1 (3)
	II	-48.0 (3)	-39.1 (3)	0.1 (4)	-27.4 (4)	151.9 (3)

* Two crystallographically independent molecules in the asymmetric unit.

† Coordinates for structure K are not available.

‡ I, dehydro-Phe at the (i + 1) position; II, dehydro-Phe at the (i + 2) position.

the (i + 2) position. If the dehydro-Phe occurs at the (i + 1) position (structures C, D), the values of φ and ψ are centred around -60 and 120° respectively. On the other hand, in a free dehydro-Phe residue and its derivatives (structures A, B) where only φ can be defined, we observe that the values of φ are close either to -60 or 80°. This suggests a marked preference of dehydro-Phe for the two conformations with φ , ψ values of either -60, 120 or 80, 0°. As seen from Table 7 and Fig. 4 (structures L, M), if the dehydro-Phe occurs consecutively in a sequence, the backbone bends at every C^α atom in reverse directions and the side chains are disposed on opposite sides. The resulting structures are alternating left- and right-handed α -helix conformations as suggested by the values of φ and ψ .

A detailed examination of the values of φ and ψ in some sequences indicates systematic deviations of the β -turn conformation from that defined by Venkatachalam (1968) thus suggesting that the steric effects of the neighbouring side chains on the dehydro-Phe residue influence the backbone bending

significantly. It is noteworthy that the largest deviations in the values of φ and ψ occur in *N*-Boc-L-Phe-dehydro-Phe-L-Val-OCH₃, structure *I* (Singh, Haridas, Chauhan & Kumar, 1987), while the smallest deviations are in *N*-Boc-L-Gly-dehydro-Phe-NHCH₃, structure *E* (Singh, Narula, Chauhan & Kaur, 1989). It is clear from the above sequences that the maximum steric effects involving the side chains are present in the former and the minimum steric constraints are in the latter. In fact, an examination of the sequences in Table 7 indicates a relationship between the observed deviations in the values of φ and ψ and the steric effects from the side chains adjacent to the dehydro residue. Therefore, the predictable contribution of these effects can be exploited for fine tuning in the accurate design of structures. A plot of the main-chain torsion angles φ and ψ for the dehydro-Phe residue in 10 structures (*C*-*J*, *L*, *M*) in which φ and ψ can be fully defined is illustrated in Fig. 3. The conformations of the peptides containing dehydro-Phe residues are shown in Fig. 4.

As seen from Table 7, the torsion angles of the side chain of dehydro-Phe in various peptides are very similar. The values of the side-chain torsion angles χ^1 , $\chi^{2,1}$ and $\chi^{2,2}$ suggest that the dehydro-Phe side chain is planar.

Conformation of the backbone with a dehydro-Leu residue

As seen from Table 8, in the structures *O* and *N* where only φ can be defined, the values of φ are close either to -60 or -80° . In the last two peptides, the dehydro-Leu is placed at the $(i+2)$ position of the β -turn and the values of φ and ψ lie in the vicinity of 80 and 0° . It is indeed an important

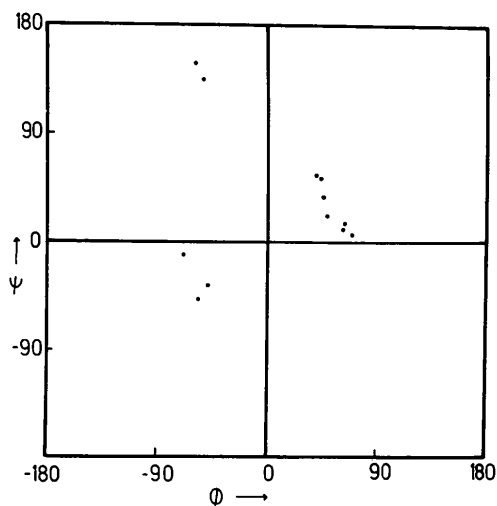


Fig. 3. Plot of main-chain torsion angles φ , ψ experimentally determined for dehydro-Phe in structures *C*-*J*, *L* and *M*.

feature of dehydro-Leu that, as in dehydro-Phe, the inclusion of a dehydro residue in a sequence forces the backbone to adopt a β -turn II conformation. A comparison of structures *G* and *I* with structures *P* and *Q* respectively reveals yet another significant feature: the steric effects caused by the dehydro-Phe residue are much more pronounced than those generated by the dehydro-Leu residue. This provides a useful variation of conformation through the introduction of different dehydro residues. The conformations of the side chain of dehydro-Leu in various sequences are similar. The values of torsion angles χ^1 , $\chi^{2,1}$ and $\chi^{2,2}$ are centred around 0 , -110 and 130° respectively which are very different from those found in a saturated Leu residue (Benedetti, 1977). The conformations of peptides containing dehydro-Leu are shown in Fig. 5.

As seen from Table 6, the value of φ in dehydro-Ala is $179.2(3)^\circ$. As has been mentioned before, the introduction of a dehydro residue results in substantial bending of the backbone. The dehydro-Ala side chain has only a $-\text{CH}_2$ side group which does not introduce significant steric effects that would cause the bending of the backbone. Presumably, the backbone adopts an extended conformation (Fig. 6). However, the value of the $\text{N}-\text{C}^\alpha-\text{C}'$ bond angle of $110.8(3)^\circ$ is considerably smaller than the corresponding values in dehydro-Phe and dehydro-Leu residues. This seems to account for the small steric effects which might be present in dehydro-Ala and which, consequently, leave the backbone unaffected.

There is only one example of a dehydro-Pro residue which is given in sequence *S*. The values of the side-chain torsion angles χ^1 , χ^2 , χ^3 , χ^4 and θ° in proline suggest that the pyrrolidine ring adopts a *C'*-*exo* conformation (Kawai, Butsugan, Fukuyama & Taga, 1987). The structure of dehydro-Pro is shown in Fig. 7.

It is noteworthy that the peptide bonds are *trans* in all these structures ($\omega \approx 180^\circ$). The most significant effects caused by the introduction of dehydro residues are steric effects which are generated due to the flattening of the dehydro residue. As stated above, these effects lead to predictable peptide conformations.

NMR studies

¹H NMR studies have also been carried out on various peptides containing dehydro residues (Bach & Gierasch, 1985, 1986; Imazu, Shimohigashi, Kodama, Sakaguchi, Waki, Kato & Izumiya, 1988; Castiglione-Morelli, Tancredi, Trivellone, Balboni, Marastoni, Salvadori & Tomatis, 1988; Castiglione-Morelli, Saviano, Temussi, Balboni, Salvadori & Tomatis, 1989). These studies indicated possible β -turn conformations. The energy calculations made

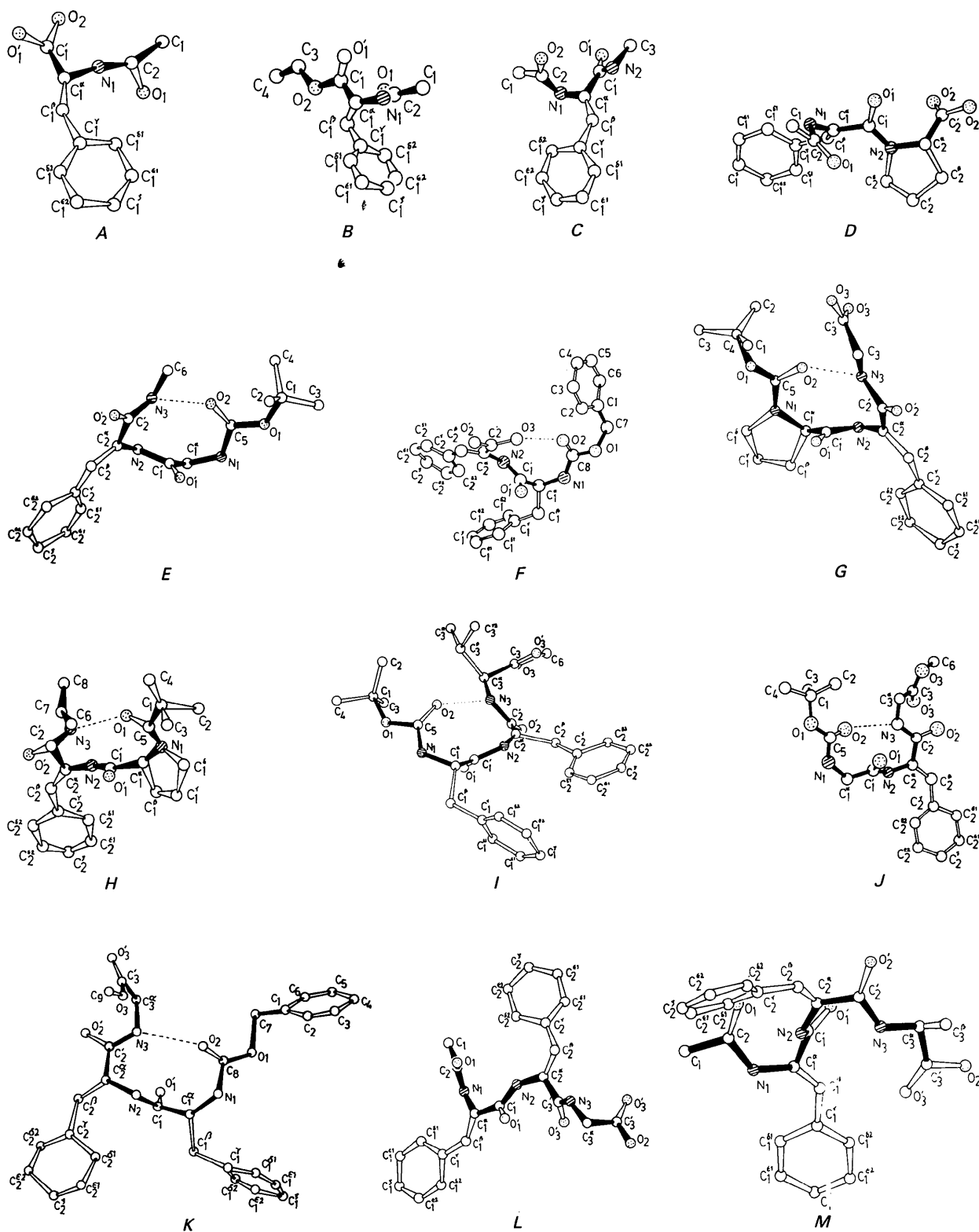


Fig. 4. Conformations of peptides containing the dehydro-Phe residue: *A*, *N*-Ac- Δ Phe-OH; *B*, *N*-Ac- Δ Phe-OCH₂CH₃; *C*, *N*-Ac- Δ Phe-NHCH₃; *D*, *N*-Ac- Δ Phe-L-Pro-OH; *E*, *N*-Boc-L-Gly- Δ Phe-NHCH₃; *F*, *N*-phenylmethoxycarbonyl-L-Phe- Δ Phe-OH; *G*, *N*-Boc-L-Pro- Δ Phe-L-Gly-OH; *H*, *N*-Bu-CO-L-Pro- Δ Phe-NHCH(CH₃)₂; *I*, *N*-Boc-L-Phe- Δ Phe-L-Val-OCH₃; *J*, *N*-Boc-L-Gly- Δ Phe-L-Gly-OCH₃; *K*, *N*-(carbobenzoyl)oxy-L-Phe- Δ Phe-L-Gly-OCH₃; *L*, *N*-Ac- Δ Phe- Δ Phe-L-Gly-OH; *M*, *N*-Ac- Δ Phe- Δ Phe-L-Ala-OH.

Table 8. Torsion angles ($^{\circ}$) of the dehydro-Leu moiety in various peptides

N^*		φ	ψ	χ^1	$\chi^{2,1}$	$\chi^{2,2}$
I	I	-56.14 (8)	—	-6.39 (1)	-115.82 (7)	121.03 (6)
	II	-54.46 (8)	—	-5.91 (10)	-124.02 (6)	111.78 (7)
O*	I	-98 (1)	—	-5 (2)	-111 (2)	126 (2)
	II	-67 (1)	—	-16 (2)	-110 (2)	133 (2)
P		74 (2)	8 (2)	12 (2)	-112 (2)	136 (2)
Q		54.5 (9)	31.1 (10)	2.7 (13)	-107.3 (11)	131.3 (10)

* Two crystallographically independent molecules in the asymmetric unit.

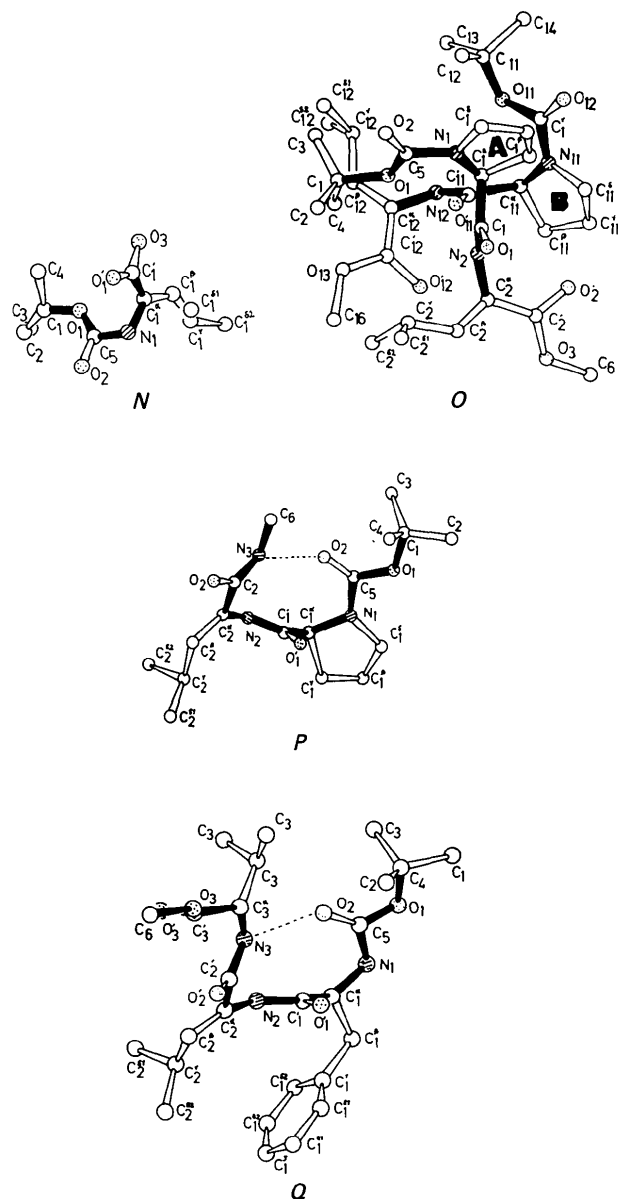


Fig. 5. Conformations of peptides containing the dehydro-Leu residue: *N*, *N*-Boc- Δ Leu-OH; *O*, *N*-Boc-L-Pro- Δ Leu-OCH₃; *P*, *N*-Boc-L-Pro- Δ Leu-NHCH₃; *Q*, *N*-Boc-L-Phe- Δ Leu-L-Val-OCH₃.

by Ajo, Busetti & Granozzi (1982) on dehydro-Phe also suggest low-energy regions at the same φ , ψ values (Fig. 8) as obtained by X-ray diffraction experiments. Therefore, the results of NMR and theoretical calculations also tend to support the conclusions drawn using X-ray diffraction results.

Concluding remarks

The findings of this study can be broadly stated as follows:

(1) Dehydro residues are essentially planar moieties.

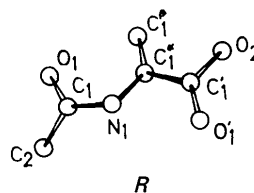


Fig. 6. Conformation of *N*-Ac- Δ Ala-OH.

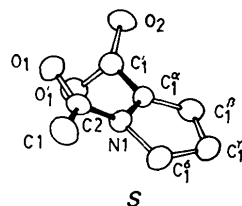


Fig. 7. Conformation of *N*-Ac- Δ Pro-OH.

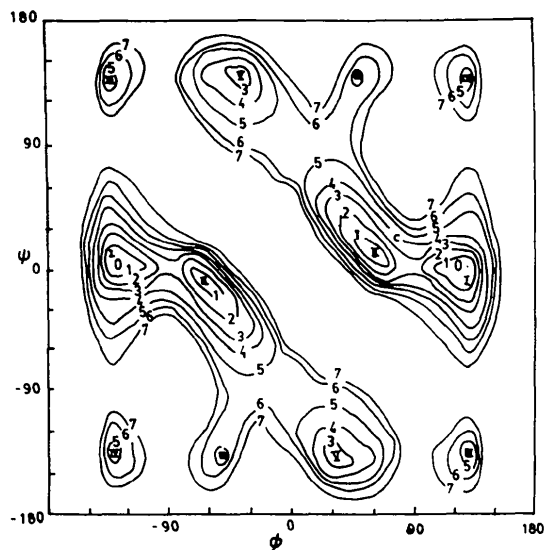


Fig. 8. Results of energy calculations in a φ , ψ plot for Δ Phe. Reprinted with permission from Ajo, Busetti & Granozzi [*Tetrahedron* (1982), **38**, 3329-3334]. © 1982 Pergamon Press plc.

(2) The single-bond distance of 1.54 Å between C^α and C^β shortens to a C^α=C^β double-bond distance of about 1.331 (2) Å.

(3) The backbone bends at the C^α atom of the dehydro residue as a result of unfavourable steric interactions between the atoms of the backbone and those of the side chains.

(4) Similar steric effects are caused by the side-chain atoms of the dehydro residues and those of the neighbouring side chains. This contributes further to the folding of the backbone.

(5) As a result of the bending of the backbone, an intramolecular hydrogen bond is observed between CO of the *i*th residue and NH of the (*i* + 3)th residue, further stabilizing the β-bend.

(6) A typical β-bend of the type II is formed if dehydro-Phe or dehydro-Leu is introduced at the (*i* + 1) or at the (*i* + 2) position in a peptide sequence of at least four residues.

(7) The backbone adopts a structure with an alternating left- and right-handed α-helix if consecutive residues are dehydro residues.

(8) The β-turn II conformations observed in these peptides have φ, ψ values which extend over a significant range. The spread is due to the rigid nature of the side chain of the dehydro residue, as a result of which the backbone is more easily influenced by interactions between the side chains.

This study suggests more definite principles for designing specific peptide structures than the currently available approaches. The judicious placement of dehydro residues can, therefore, lead to the design of specific structures.

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