CH_3OH) (a single stereoisomer as assayed by ^{13}C NMR).[13] The acetylenic compounds of type **6** serve as common intermediates for the general synthesis of the PG family.[1,3b] With this highly efficient chemical operation secured, PGI_2 is now obtainable in only five steps starting from the chiral cyclopentenone **1**.[16]

Registry No. **1**, 61305-35-9; **2a** (lithio derivative), 41138-68-5; **2b** (lithio derivative), 96038-40-3; **3**, 64493-06-7; **4**, 31776-12-2; **5a**, 66602-10-6; **5a** (PGE_1 analogue), 86982-75-4; **5b**, 95935-97-0; **6**, 59895-16-8; $I(CH_2)_6COOCH_3$, 38315-25-2.

(16) Suzuki, M.; Yanagisawa, A.; Noyori, R. *Tetrahedron Lett.* **1983**, *24*, 1187.

Dehydrophenylalanine as the $i + 2$th Residue of a β Turn: Synthesis and Conformational Analysis of *cyclo*(Gly-Pro-Δ^z-Phe-D-Ala-Pro) and *cyclo*(Gly-Pro-D-Phe-D-Ala-Pro)

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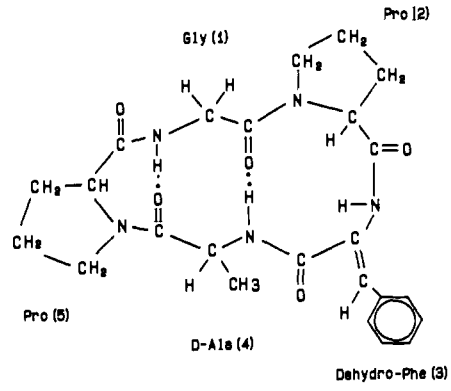
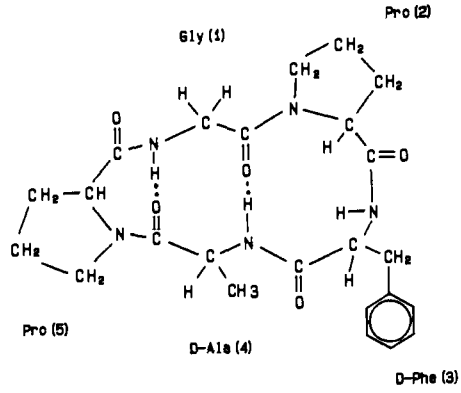
Analogues of several biologically active peptides, in which *trans*-α,β-didehydrophenylalanine (Δ^z-Phe) is substituted for phenylalanine, exhibit high potency[1-3] and increased resistance to chymotrypsin degradation.[1] However, in other examples[4] the Δ^z-Phe-containing analogue is markedly less active than the Phe-containing peptide. While the conformational behavior of a Δ^z-Phe, with π-bonding between C^α and C^β, is expected to differ significantly from that of (saturated) Phe, there is as yet no clear understanding of its influence on the available conformations of a peptide.

In both X-ray diffraction analyses and conformational energy calculations of Δ^z-Phe-containing peptides the ϕ angle of the dehydro residue is frequently near 60° and ψ near 0°.[5] For example, the X-ray crystal structure of *N*-acetyl-Δ^z-Phe has ϕ = 72° and ψ = 13°;[6] the X-ray crystal structure of *N*-pivaloyl-Pro-Δ^z-Phe-methylamide has ϕ = 63° and ψ = 10° for Δ^z-Phe;[7] energy calculations on *N*-acetyl-Δ^z-Phe-methylamide show an energy minimum at ϕ = 60° and ψ = 10°.[8] The similarity of the preferred Δ^z-Phe conformation to that taken up by a residue in the $i + 2$ position of a type II β turn is noteworthy: average ϕ and ψ values in type II β turns (from X-ray data) are 80° and 0°, respectively, for the $i + 2$ position.[9] Substitution of Δ^z-Phe for such a residue in a peptide may result in a conformationally homologous *dehydropeptide*.

To test this hypothesis we have synthesized two cyclic pentapeptides: *cyclo*(Gly^1-Pro^2-Δ^z-Phe^3-D-Ala^4-Pro^5) I (the cyclic dehydropeptide) and *cyclo*(Gly^1-Pro^2-D-Phe^3-D-Ala^4-Pro^5) II (the cyclic peptide). We anticipated from previous work[10-12] that I would favor a Gly-Pro-D-Phe-D-Ala type II β turn, i.e., with D-Phe in position $i + 2$ of the turn and with a D-Ala-Pro-Gly γ turn (see below). We present evidence that it does. Furthermore, substitution of Δ^z-Phe for D-Phe (in peptide I) causes very little conformational change, in keeping with the above hypothesis.



(I)



(II)

The cyclic peptide II was synthesized by methods previously reported,[10] including cyclization of the pentapeptide *p*-nitrophenyl ester (yield, 37%). An unsaturated azlactone was prepared from Boc-Pro-DL-β-phenyl-Ser-OH by the modified Bergmann synthesis[13] and was coupled with H-D-Ala-Pro-Gly-OMe giving a Δ^z-Phe-containing pentapeptide which was then cyclized as the *p*-nitrophenyl ester (yield, 5%). Since the cyclization conditions were the same for I and II, these different yields reflect the relative ease of forming cyclic product; the required folded conformation may be less accessible to the dehydropeptide. Both I and II are pure by thin-layer and high-performance liquid chromatography, and their monomeric character was confirmed by chemical ionization mass spectroscopy.[14]

^1H and ^{13}C nuclear magnetic resonance (NMR) data (Figures 1B and 2B) support the proposed conformation of the cyclic peptide II. The resonances of the D-Ala and Gly NH's occur at 7.83 and 7.78 ppm, respectively, typical of NH's involved in intramolecular hydrogen bonds;[9,10c] by comparison, the D-Phe NH resonates at higher field (5.89 ppm) as expected for a non-hydrogen-bonded NH in a solvent like $CDCl_3$, at high dilution (21 mM).[9,10c] The D-Ala and Gly NH's also show reduced temper-

(1) English, M. L.; Stammer, C. H. *Biochem. Biophys. Res. Commun.* **1978**, *83*, 1464–1467.
(2) Chipkin, R. E.; Stewart, J. M.; Stammer, C. H. *Biochem. Biophys. Res. Commun.* **1979**, *87*, 890–895.
(3) English, M. L.; Stammer, C. H. *Biochem. Biophys. Res. Commun.* **1978**, *85*, 780–782.
(4) Brady, S. F.; Cochran, D. W.; Nutt, R. F.; Holly, F. W.; Bennett, C. D.; Paleveda, W. J.; Curley, P. E.; Arison, B. H.; Saperstein, R.; Veber, D. F. *Int. J. Peptide Protein Res.* **1984**, *23*, 212–222.
(5) Ajo, D.; Granozzi, G.; Tondello, E. *Biopolymers* **1980**, *19*, 469–475.
(6) Ajo, D.; Casarin, M.; Granozzi, G.; Busett, V. *Tetrahedron* **1981**, *37*, 3507–3512.
(7) Aubry, A.; Allier, F.; Boussard, G.; Marraud, M. Proceedings of the International Forum on Peptides, Le Cap D'Agde-France, Sept 24–28, 1984, in press.
(8) Ajo, D.; Casarin, M.; Granozzi, G. *J. Mol. Struct.* **1982**, *86*, 297–300.
(9) Smith, J. A.; Pease, L. G. *CRC Crit. Rev. Biochem.* **1980**, *8*, 315–399.
(10) (a) Pease, L. G.; Watson, C. *J. Am. Chem. Soc.* **1978**, *100*, 1279–1286. (b) Pease, L. G.; Niu, C. H.; Zimmermann, G. *J. Am. Chem. Soc.* **1979**, *101*, 184–191. (c) Pease, L. G. "Peptides: Structure and Biological Function"; Gross, E., Meienhofer, J., Eds.; Pierce Chemical Co.: Rockford, IL, 1979; pp 197–200.
(11) Bach, A. C., II; Bothner-By, A. A.; Gierasch, L. M. *J. Am. Chem. Soc.* **1982**, *104*, 572–576.
(12) Karle, I. L. *J. Am. Chem. Soc.* **1978**, *100*, 1286–1289.
(13) Konno, S.; Stammer, C. H. *Int. J. Peptide Protein Res.* **1978**, *12*, 222–231.
(14) Precise Mass: I, 468.226 ($C_{24}H_{27}N_5O_5$ + H)$^+$; II, 470.241 ($C_{24}H_{29}N_5O_5$ + H)$^+$.

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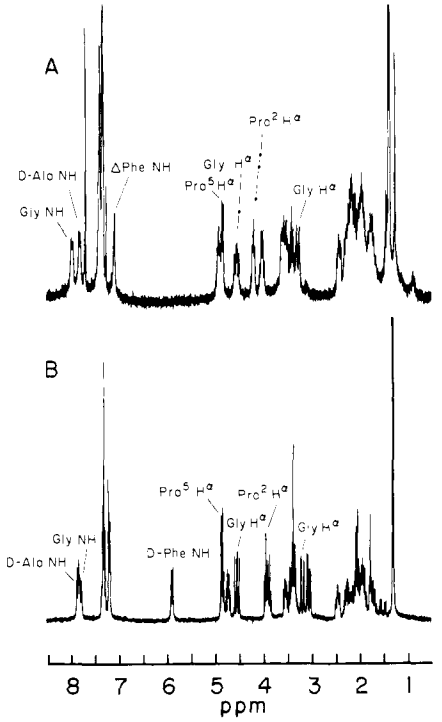

Figure 1. ^{1}H NMR spectra (250 MHz): (A) *cyclo*(Gly^1-Pro^2-Δ^z-Phe^3-D-Ala^4-Pro^5) in $CDCl_3$, 64 scans, concentration 11 mM; (B) *cyclo*(Gly^1-Pro^2-D-Phe^3-D-Ala^4-Pro^5) in $CDCl_3$, 16 scans, concentration 21 mM. All spectra were recorded at ambient temperature.

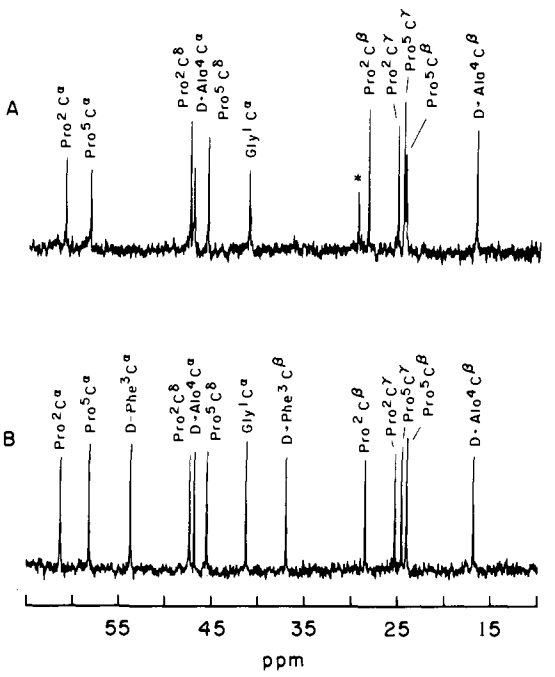

Figure 2. ^{13}C NMR spectra (62.9 MHz): (A) *cyclo*(Gly^1-Pro^2-Δ^z-Phe^3-D-Ala^4-Pro^5) in $CDCl_3$, 82000 scans, concentration 11 mM; (B) *cyclo*(Gly^1-Pro^2-D-Phe^3-D-Ala^4-Pro^5) in $CDCl_3$, 48000 scans, concentration 21 mM. All spectra were recorded at ambient temperature. The starred resonance in (A) is due to an acetone impurity.

ature coefficients (3.4 and 1.1 ppb/deg, respectively) relative to the exposed D-Phe NH (8.2 ppb/deg) at higher peptide concentrations (170 mM in $CDCl_3$ with 4% v/v dimethyl-d_6 sulfoxide).[15] The Pro^5 H$^\alpha$ resonance is shifted strongly to low field (4.85 ppm) and appears as a doublet with one large and one small coupling constant to the β protons, while the Pro^2 H$^\alpha$ appears as a triplet, i.e., nearly equal coupling constants at 3.94 ppm.[16] The downfield position and doublet appearance are typical of prolines occurring as the $i + 1$th residue of γ turn.[9,10] One of the proline C$^\beta$ resonances shows an upfield shift indicative of a γ turn (C$^\beta$ at 24.4 ppm, by comparison with the more usual 28–30 ppm).[9,10] The Pro carbon chemical shifts also confirm that II adopts an all-trans conformation.[17] These constraints uniquely determine the conformation of II.

Substitution of Δ^z-Phe for D-Phe (peptide I) causes very little change in the NMR spectral parameters (Figures 1 and 2): the D-Ala and Gly NH's are at low field (7.82 and 7.98 ppm) and have small temperature coefficients (2.1 and 1.7 ppb/deg) relative to the Δ^z-Phe NH (7.15 ppm, 4.6 ppb/deg); Pro^5 has a downfield "doublet" H$^\alpha$ resonance (4.85 ppm) and Pro^2 has a more usual position (4.25 ppm) and distorted triplet shape;[16] the proline β and γ carbon chemical shifts are nearly the same as in the peptide. These highly diagnostic indicators of backbone conformation argue strongly for the retention of the β,γ-turn conformation in the dehydropeptide. Even more striking are the detailed aspects of the ^{1}H NMR spectra: the chemical shifts vary little between I and II despite the presence of the Δ^z-Phe residue.

Thus, a Δ^z-Phe residue can be substituted for a saturated residue with minimal perturbation to a peptide's backbone conformation if it is placed in the $i + 2$ position of a type II β turn where ϕ and ψ angles are generally near 80° and 0°.[9] In a bioactive peptide, an analogous substitution of Δ^z-Phe should not cause loss of activity, provided that (1) the available backbone conformation coincides with the functional conformation and (2) the side-chain conformation at the substituted site is unchanged or not essential for activity.

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Registry No. I, 96109-55-6; II, 96095-88-4.

(15) Stevens, E. S.; Sugawara, N.; Bonora, G. M.; Toniolo, C. *J. Am. Chem. Soc.* **1980**, *102*, 7048–7050.
(16) Assignment of Pro^2 in both I and II is based upon observation of a nuclear Overhauser enhancement (NOE) between its H$^\alpha$ and the D-Phe or Δ^z-Phe NH.
(17) Dorman, D. E.; Bovey, F. A. *J. Org. Chem.* **1973**, *38*, 2379–2383.

Crystallization-Induced Changes in Protein Structure Observed by Infrared Spectroscopy of Carbon Monoxide Liganded to Human Hemoglobins A and Zurich

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The relationship of a protein structure obtained by crystallography to the in vivo solution structure is of great interest to the study of protein structure and function. Upon crystallization, the structure of a protein molecule may change due to crystal lattice forces and different hydration levels.[2] With hemeproteins, ligand infrared spectra can provide a sensitive probe for monitoring ligand site structures in both crystals and solutions.[3,4] The spectra for CO, CN^-, N_3^-, NO, and O_2 as ligands have been observed for several hemeproteins in solution and provide evidence that multiple ligand site structures (conformers) are present.[5] IR

(1) Present address: Bioproducts-Central Research, Dow Chemical USA, Midland, MI 48640.
(2) Scanion, W. J.; Eisenberg, D. *J. Phys. Chem.* **1981**, *85*, 3251–3256.
(3) Caughey, W. S. In "Methods for Determining Metal Ion Environments in Proteins: Structure and Function of Metalloproteins"; Darnell, D. W., Wilkins, R. G., Eds.; Elsevier: New York, 1980; pp 95–115.
(4) Makinen, M. W.; Houtchens, R. A.; Caughey, W. S. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 6042–6046.

0002-7863/85/1507-3350$01.50/0