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Structure and conformation of the monohydrate of N-t-boc-tyrosyl-proline (Boc-Tyr-Pro·H_2O)

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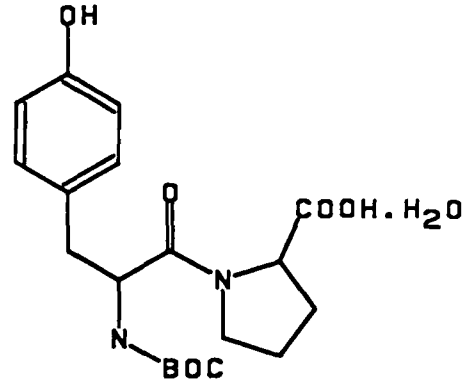
The structure and conformation of the monohydrate of N-t-boc-tyrosyl-proline (Boc-Tyr-Pro·H_2O) ($C_{19}H_{26}O_6N_2 \cdot H_2O$) has been investigated with X-ray crystallographic and spectroscopic methods. Boc-Tyr-Pro crystallized in an extended *trans* conformation in the space group $P2_12_12_1$ with cell dimensions $a = 8.566(1)$, $b = 9.996(1)$, $c = 24.734(1)$. The conformation of Boc-Tyr-Pro reflex α-helix type prolines. Three intermolecular hydrogen bonds are observed. Crystal water is involved in two hydrogen bonds (to the hydroxyl group of the C-terminal of the proline residue; to the carbonyl group of the t-Boc functionality) while the hydroxyl group of the tyrosyl residue (to the carbonyl group of the amide bond) is involved in one hydrogen bond. The puckering mode of the pyrrolidine ring of the proline residue is similar to what has been previously observed for other proline-containing peptides. *Cis-trans* isomerism is observed in the NMR spectra of Boc-Tyr-Pro with a predominance for the extended side chain for the tyrosyl residue.

KEY WORDS: Dipeptides, proline, NMR, hydrogen bonds.

Introduction

There has been an ever increasing attention in the synthesis, chemistry, and biological properties of proline containing peptides and proteins. First, proline is a unique imino acid in so far as it is the only residue which leads to an N-alkylamide bond when incorporated into a peptide via natural biochemical pathways. Second, proline residues impose significant conformational restraints on peptides and proteins[1-3] and thirdly the *cis-trans* isomerism of the N-alkylamide bond involving the amino group of proline has been implicated in the biological activity of peptides.[4,5] The current study, on the structure and conformation of the monohydrate of tert-butyloxycarbonyl-tyrosyl-proline (Boc-Tyr-Pro·H_2O), forms part of our interest into small proline containing peptides related to the μ-opioid receptor agonist, morphiceptin (Tyr-Pro-Phe-Pro-NH_2) and its fragments.[6] We recently reported the synthesis, conformational and spectroscopic properties of the cyclic dipeptide, cyclo(Tyr-Pro)[7] and the tripeptide, Boc-Pro-Phe-Pro.[8]



Experimental

Synthesis of Boc-Tyr-Pro·H_2O

All reagents and solvents were of reagent grade and used without further purification. N-t-Boc-S-Tyr-hy-

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droxysuccinic imide ester (4 g, 10.57 mmol) and 1,2-dimethoxy ethane (30 mL) were added to a solution of S-Pro-OH (1,217 g, 10.57 mmol) and sodiumbicarbonate (0.888 g, 10.57 mmol) in water (10 mL) and stirred for 12 h. After the removal of the solvent in vacuo, a saturated sodium chloride solution (30 mL) was added to the crude syrup and the pH was adjusted to a value of 2 hydrochloric acid (5%). The product was extracted with ethylacetate (3 × 30 mL) which was removed in vacuo. Recrystallization from ethanol-acetone-ethylacetate-n-hexane yielded Boc-Tyr-Pro·H_2O (2.47 g, 59%, m.p. 102–103°C) [*Rf* = 0.46; Chloroform-methanol-acetic acid (19:3:1)].

X-ray analysis

Colorless crystals of Boc-Tyr-Pro·H_2O ($C_{19}H_{28}O_7N_2$) crystallized from ethanol-acetone-ethylacetate-*n*-hexane in the space group $P2_12_12_1$. The crystal selected for data collection had the dimensions 0.17 × 0.27 × 0.50 mm. Reflections (3500) were collected with their indices being *h* 0:12, *k* 0:14, *l* 0:34.

All diffraction measurements were performed at room temperature and data collected with an Enraf-Nonius CAD4 diffractometer using monochromated Mo-K_α radiation. The lattice constants were obtained from a least squares fit of 25 centered reflections (7° ≤ θ ≤ 17°) and are listed with other relevant crystal data in Table 1. The data were corrected for Lorentz and polarization effects. No absorption corrections were applied. Intensity checks were carried out every hour and an orientation control every 200 reflections. Three standard reflections were used to check orientation and crystal stability at regular intervals, and the decay during data collection was 0.3% (not corrected). The structure was solved by direct methods and refined anisotropically using a full matrix method ($1/\sigma^2F$-weights) using ShelX76.[9]

All hydrogen atoms, except the experimentally located and refined H15, H27, H28A and H28B were placed in calculated positions and all hydrogen atoms were included in the refinement with a common isotropic temperature factor that converged to U = 0.117 (4). Fractional coordinates and equivalent thermal factors for Boc-Tyr-Pro·H_2O are listed in Table 2. The relevant torsion angles are listed in Table 3.

Spectroscopic analyses

^1H Proton (300 MHz) and ^{13}C carbon (75 MHz) spectra were recorded on a Bruker Am-300 spectrometer, with DMSO as solvent and TMS as internal standard. Hetcor and Cosy spectra were recorded to assist with the ^1H and ^{13}C assignments. Infrared spectra were

Table 1. Crystal data of Boc-Tyr-Pro·H_2O

Formula $C_{19}H_{26}O_6N_2 \cdot H_2O$
Space group $P2_12_12_1$
a = 8.566(1) Å
b = 9.996(1) Å
c = 24.734(1) Å
V = 2117.9 Å^3
Z = 4
D = 1.242 g cm^{-3}
λ Mo-K_α = 0.7107 Å
μ = 0.59 cm^{-1}
$F(000)$ = 848
Scan type (ω:2θ) = 1:1
Scan range (θ)° = 3 ≤ θ ≤ 30
Scan speed (variable. deg. min^{-1}) = Max 5.49
Scan angle (ω + 0.34 tan θ°) = 0.45
Aperture size (mm) = 1.3 × 4.0
R_w = 0.035
R = 0.075 for 2074 unique observed reflections ($I > 1\ \sigma I$)

Table 2. Fractional coordinates (×10^4) and equivalent thermal factors (×10^3 Å^2) Boc-Tyr-Pro·H_2O

	x/a	*y/b*	*z/c*	U_{eq}
C(1)	10468(8)	4623(6)	2812(2)	99(2)
C(2)	10390(8)	3497(5)	1909(2)	103(2)
C(3)	12017(6)	5577(7)	2043(3)	105(2)
C(4)	10551(7)	4809(5)	2210(2)	72(2)
O(5)	9153(4)	5646(3)	2096(1)	61(1)
C(6)	8792(6)	6006(4)	1592(2)	50(1)
O(7)	9554(4)	5823(3)	1193(1)	66(1)
N(8)	7378(4)	6636(3)	1587(1)	53(1)
C(9)	6642(5)	6870(4)	1060(2)	47(1)
C(10)	5040(5)	7538(4)	1155(2)	56(1)
C(11)	5159(6)	8976(4)	1365(2)	49(1)
C(12)	4475(6)	9341(5)	1835(2)	72(2)
C(13)	4497(7)	10669(6)	2008(2)	91(2)
C(14)	5232(8)	11642(5)	1700(2)	70(2)
O(15)	5209(6)	12928(3)	1882(1)	100(1)
C(16)	5957(7)	11266(5)	1234(2)	69(1)
C(17)	5907(6)	9946(5)	1066(2)	65(1)
C(18)	6327(5)	5530(5)	780(2)	49(1)
O(19)	6034(4)	4515(3)	1044(1)	68(1)
N(20)	6272(4)	5556(4)	239(1)	46(1)
C(21)	6581(6)	6673(4)	−128(2)	62(1)
C(22)	6058(7)	6162(5)	−672(2)	80(2)
C(23)	6219(7)	4684(6)	−639(2)	80(2)
C(24)	5853(5)	4331(5)	−48(2)	53(1)
C(25)	4130(6)	3952(5)	24(2)	58(1)
O(26)	3659(4)	2852(3)	−69(2)	89(1)
O(27)	3239(4)	4958(4)	173(2)	67(1)
O(28)	10297(4)	4505(4)	263(2)	81(1)

$U_{eq} = \frac{1}{3} \Sigma_{ij} \cdot i^* \cdot j$

Table 3. The relevant torsion angles of Boc-Tyr-Pro·H_2O

Torsion Angles/°		
C6–N8–C9–C18	ϕ_1	−61.4(5)
N8–C9–C18–N20	ψ_1	153.7(4)
C9–C18–N20–C24	ω_1	175.2(4)
N8–C9–C10–C11	χ_1	−69.3(5)
C9–C10–C11–C12	χ_1	122.9(5)
C9–C10–C11–C17	χ_1	−60.2(6)
C18–N20–C24–C25	ϕ_2	−70.4(5)
N20–C24–C23–C22	χ_2	26.6(5)
C24–C23–C22–C21	χ_2	−33.3(6)
C23–C22–C21–N20	χ_2	26.4(6)
C22–C21–N20–C24	χ_2	−9.7(5)
C21–N20–C24–C23	θ	−10.3(5)

recorded on a Perkin-Elmer Model 1600 FTIR spectrophotometer as KBr disks. Fast atom bombardment (FAB) mass spectrum of Boc-Tyr-Pro·H_2O dissolved in DMSO with 3-nitrobenzyl alcohol as matrix was obtained on a VG-7070E spectrometer.

Results and discussion

X-ray analysis

Figure 1 shows the ORTEP drawing of Boc-Tyr-Pro·H_2O complete with the numbering scheme and the X-ray data appears in Table 2. The torsion angles of the backbone and relevant side chains of Boc-Tyr-Pro·H_2O appear in Table 3.

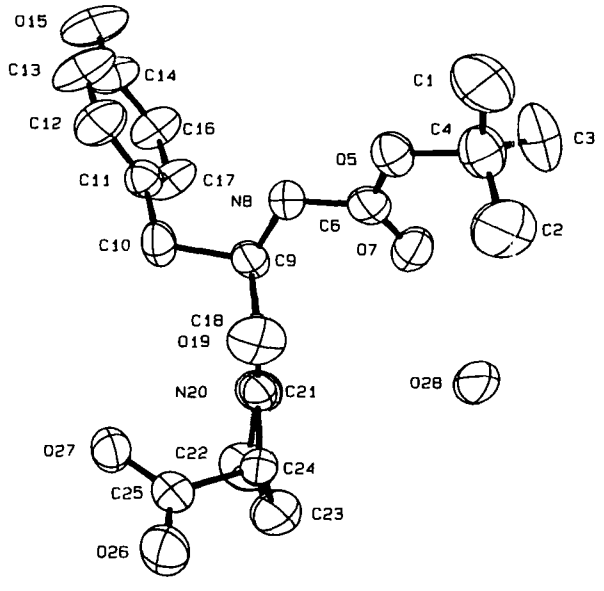

Fig. 1. ORTEP view of Boc-Tyr-Pro·H_2O.

The amide bond torsion angle (ω_1 = 175.2°) indicates a near trans conformation for the dipeptide. The positive value of ψ_1(153.7°) indicates that the dipeptide belongs to the α-helix type of proline compounds.[2,10] We previously reported that the proline containing tripeptide (Pro-Phe-Pro) adopted both α-helix type and collagen type conformations by crystallizing into two distinctly different conformers.[8] The puckering mode of the proline residue of Boc-Tyr-Pro assumes a half-chair conformation of type A which can be described as a ${}^{\gamma}_{\beta}T$ conformation, with the C^{β} atom positioned endo and C^{γ} position exo relative to the carboxamide gorup.[2,11] The same puckering mode was also observed for the pyrrolidine rings of the cyclodipeptide, cyclo(Phe-Pro)[12] and the ^{1}Pro residue of the conformer 2 of the tripeptide Boc-Pro-Phe-Pro.[8] A distinctly different puckering mode (${}^{\beta}_{\gamma}T$; C_s-envelope)[13–15] was adopted by the ^{1}Pro (conformer 1), ^{3}Pro (conformer 1), and ^{3}Pro (conformer 2) residues of Boc-Pro-Phe-Pro.[8] Three molecular hydrogen bonds of the intermolecular type were observed in the crystal structure for the monohydrate of Boc-Tyr-Pro (Table 4). Crystal water is involved in a hydrogen bond to the carboxy terminal (O27–H27-----O28) and to the carbonyl oxygen of the t-Boc group (O28–H28-----O7). The phenolic hydroxyl group of the tyrosyl residue is hydrogen bonded to the carbonyl oxygen of amide group of an adjacent molecule (O15–H15-----O19). The role of the crystal water in stabilizing the dipeptide in this conformation is not clear and the X-ray structure of the anhydrous compound or any other solvate form could assist in determining its influence.

Spectroscopic analyses

The mass spectrum of Boc-Tyr-Pro·H_2O shows a parent ion peak at *m/z* 379, i.e., the expected anhydrate form. The characteristic tyrosyl side chain cleavage yielding the fragmention *m/z* 107 [C_7H_7O] is the highest observed fragment ion in the mass spectrum.[16] The NMR data of Boc-Tyr-Pro·H_2O clearly indicate the presence of the *cis*(~15)-*trans*(~85) isomerism (well known for proline containing peptides.[17] A predomi-

Table 4. Hydrogen bonding schemes for Boc-Tyr-Pro·H_2O. D-donor, H-hydrogen, A-acceptor

D–H···A	D–A	H···A	Angle D–H···A
027–H27···O28	2.570 Å	1.814 Å	172.1°
028–H28···O7	2.727 Å	1.731 Å	166.7°
015–H15···O19	2.705 Å	1.816 Å	170.0°

Table 5. ^{13}C NMR data of Boc-Tyr-Pro·H_2O[a]

Carbon Atom	PPM
Pro-γ	24.6(t)
$C(\underline{C}H_3)_3$	28.1(q)
Pro-β	28.5(t)
Tyr-β	35.5(t)
Pro-δ	46.3(t)
Tyr-α	54.0(d)
Pro-α	58.6(d)
$\underline{C}(CH_3)_3$	77.9(s)
Tyr−Ar	114.8(d)
Tyr−Ar	127.9(s)
Tyr−Ar	130.2(d)
Tyr−Ar	155.2(s)
Boc−C=O	155.8(s)
Tyr−C=O	170.3(s)
Pro−C=O	173.2(s)

[a] = singlet; d = doublet; t = triplet; q = quartet.

nance of the extended conformation (63%) for the Tyr side chain in solution was estimated (using Pachler's analysis;[18] which is very similar to the observed solution conformations for the cyclic depeptides, cyclo(Phe-fluoro-Pro) (72%)[19] and cyclo(Tyr-Pro) (64%)[7]. The ^{13}C NMR data appear in Table 5. The carbon chemical shift values are in close agreement with the values observed for the corresponding amino acid residues of the morphiceptin[17] and the cyclo(Tyr-Pro)[7].

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