Peptide Conformations. Crystal Structures of *tert*-Butyloxycarbonylglycyl-L-proline and Its Benzyl Ester[1a]

Richard E. Marsh,*[1b] M. R. Narasimha Murthy,[1c] and K. Venkatesan[1c]

Contribution No. 5338 from the Arthur Amos Noyes Laboratory of Chemical Physics, California Institute of Technology, Pasadena, California 91125. Received May 24, 1976

Abstract: The crystal structures of *tert*-butyloxycarbonylglycyl-L-proline and of its benzyl ester are reported. Both molecules show similar, extended conformations and there is no hint of the folded conformation that had earlier been suggested for *tert*-butyloxycarbonylglycyl-L-proline. It is shown that differences in the IR spectra of the two crystals, which had been interpreted in terms of an intramolecular hydrogen bond in the free peptide, are due instead to an *inter*molecular hydrogen bond. Both compounds crystallize in the orthorhombic space group $P2_12_12_1$, with cell dimensions a = 5.743 (2), b = 23.849 (5), and c = 10.440 (2) Å for the free peptide and a = 15.381 (3), b = 19.112 (4), and c = 6.638 (2) Å for the benzyl ester. Least-squares refinement led to R indexes of 0.049 and 0.037 for 1344 and 2273 reflections with positive net intensity.

Experimental evidence for the folding of a peptide chain into a β, or hairpin, turn has come from a variety of measurements such as crystal structure studies of proteins and peptides and NMR and optical measurements in solution.[2] This conformation seems to be favored when prolyl residues are present in the peptide sequence, presumably because of the inherent twist dictated by the steric constraints of the pyrrolidine ring. Recently, Deber[2a] carried out solid-state infrared absorption studies on a number of *t*-Boc (*tert*-butyloxycarbonyl) derivatives of prolyl-containing dipeptides and tripeptides and of their benzyl esters. These studies indicated that the carboxylic acid grouping forms a hydrogen bond to a carbonyl oxygen atom two units removed; Deber proposed that this hydrogen bond is intramolecular and, hence, that the molecular conformations of the free acids show a β turn. However, Deber noted that *inter*molecular effects could not be ruled out and that "x-ray crystallography remains the method of choice for substantiation of the postulated intramolecularly hydrogen-bonded structures".

We report here the x-ray crystallographic results on two of the compounds which Deber studied—*tert*-butyloxycarbonylglycyl-L-proline (*t*-B-G-P-OH) and its benzyl ester (*t*-B-G-P-OBz). These results show that the hydrogen bond noted by Deber for *t*-B-G-P-OH is indeed *inter*molecular and that there is no β turn in either compound.

Experimental Section

A sample of *t*-B-G-P-OH was provided by Dr. Claudio Toniolo and was recrystallized by slow evaporation of an acetone solution; a sample of *t*-B-G-P-OBz was provided by Professor E. R. Blout and was recrystallized by evaporation of an aqueous solution. Unit cell dimensions and intensity data for both compounds were collected on a Datex-automated General Electric quarter-circle diffractometer using nickel-filtered Cu Kα radiation, a scintillation detector, and pulse-height discriminator. For *t*-B-G-P-OH all reflections out to 2θ = 130° were collected at a scan speed of 2° (in 2θ) per minute and one octant was surveyed; for the benzyl ester, two octants (hkl and $hk\bar{l}$) were collected out to 2θ = 150° at a scan speed of 1° per minute. In both cases backgrounds were counted for 20 s at the scan extrema. Three check reflections were surveyed; their intensities showed no significant variation for the free acid but decayed by an average of about 10% for the benzyl ester. The measurements were corrected for this decay. Observational variances $\sigma^2(I)$ included counting statistics plus a term $(0.02S)^2$, where S is the scan count. The data set for the free acid numbered 1445, of which 96 showed net intensity less than zero and another five were subsequently assigned weights of zero; for the benzyl ester, the data (after averaging the two octants) totalled 2343 of which 68 were less than zero and two others were given zero weight. Crystal data are given in Table I.

The structure of *t*-B-G-P-OH was solved in Pasadena by a straightforward application of direct methods using the latest version of MULTAN.[3] The structure of the benzyl ester was solved in Bangalore, again with the help of MULTAN; in the latter case, however, initial attempts were unsuccessful, the various E maps always containing large, anomalous peaks. The difficulty was eventually traced to problems in scaling the data by Wilson's method,[4] the distribution of observed intensities being abnormal in the region around $\sin^2\theta/\lambda^2$ = 0.18, which corresponds to the prominent maxima in the molecular scattering curve of a benzene ring. Accordingly, spherically averaged molecular scattering factors for a benzene ring and a peptide grouping were introduced into the Wilson calculations; the distribution became normal, and a new set of E values[5] was calculated. The first-phase solution from MULTAN then led to an E map from which all of the nonhydrogen atoms were recovered.

Refinement was by least-squares minimization of the quantity $\Sigma w(F_o^2 - F_c^2)^2$, with weights w equal to $1/\sigma^2(F_o^2)$. Eventually, the parameter list included coordinates for all the atoms, anisotropic temperature coefficients for the C, N, and O atoms, isotropic temperature coefficients for the H atoms, a parameter to account for isotropic extinction effects, and a scale factor; the 253 parameters for *t*-B-G-P-OH were separated into two matrices while the 340 parameters for *t*-B-G-P-OBz were apportioned among three matrices. Final parameters and their esd's are listed in Tables II and III. The esd's in the positions of the O, N, and C atoms range from 0.002 to 0.004 Å in the free acid and from 0.0015 to 0.003 Å in the benzyl ester, the smaller values for the benzyl ester corresponding to the higher diffraction angle for which data were collected. The slower scan speed

Table I. Crystal Data

	t-Boc-Gly-Pro-OH	*t*-Boc-Gly-Pro-OBz
Formula	$C_{12}H_{20}N_2O_5$	$C_{19}H_{26}N_2O_5$
Formula wt	272.3	362.4
Space group	$P2_12_12_1$	$P2_12_12_1$
a, Å	5.743 (2)	15.381 (3)
b, Å	23.849 (5)	19.112 (4)
c, Å	10.440 (2)	6.638 (2)
V, Å^3	1430	1951
Z	4	4
D_x, g cm^{-1}	1.265	1.234
D_{obsd}, g cm^{-1}	1.26	1.214
R (n)[a]	0.049 (1344)	0.037 (2273)
Goodness of fit (m)[b]	1.39 (1440)	2.18 (2341)

[a] $\Sigma||F_o| - |F_c||/\Sigma|F_o|$ for n reflections with $F^2 > 0$. [b] $[\Sigma w(F_o^2 - F_c^2)^2/(m - s)]^{1/2}$ for m reflections with $w \neq 0$.

Table II. Coordinates ($\times 10^5$) and Anisotropic Temperature Parameters ($\times 10^4$) for the C, N, and O Atoms[a]

	x	y	z	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
				(a) *t*-B-G-P-OBz					
C(1)	69 523 (15)	52 755 (12)	−25 566 (39)	737 (14)	908 (15)	935 (17)	24 (13)	97 (15)	−84 (16)
C(2)	64 970 (17)	63 077 (16)	−45 577 (42)	834 (17)	1410 (24)	937 (19)	−280 (16)	118 (15)	400 (18)
C(3)	67 861 (15)	64 384 (14)	−8 797 (45)	757 (15)	1090 (19)	1127 (20)	−287 (15)	137 (16)	−442 (18)
C(4)	64 592 (11)	59 563 (10)	−25 304 (30)	517 (10)	728 (12)	604 (11)	−82 (9)	49 (10)	−26 (11)
C(5)	52 152 (13)	55 224 (10)	−5 946 (26)	598 (11)	620 (10)	463 (9)	−72 (10)	−15(9)	2 (9)
C(6)	38 610 (12)	51 053 (9)	8 235 (28)	598 (11)	647 (11)	477 (10)	−96 (10)	5 (9)	−5 (9)
C(7)	34 704 (12)	44 411 (9)	−138 (25)	572 (10)	564 (10)	410 (8)	−17 (9)	19 (8)	−11 (8)
C(8)	32 027 (13)	40 466 (11)	35 337 (25)	641 (12)	817 (13)	354 (8)	−121 (11)	16 (8)	−7 (9)
C(9)	27 241 (15)	33 971 (12)	42 582 (29)	933 (15)	787 (13)	422 (9)	−97 (13)	34 (11)	93 (11)
C(10)	28 320 (14)	28 693 (10)	25 841 (30)	861 (14)	627 (11)	550 (10)	−18 (11)	−72 (12)	100 (11)
C(11)	28 135 (12)	33 180 (9)	6 743 (25)	641 (11)	501 (9)	431 (8)	−20 (9)	28 (9)	−19 (8)
C(12)	18 955 (12)	34 300 (9)	−723 (25)	640 (11)	523 (9)	408 (8)	−36 (9)	53 (8)	−23 (8)
C(13)	7 664 (12)	29 409 (11)	−20 354 (32)	573 (11)	739 (12)	750 (13)	21 (11)	−53 (10)	−140 (12)
C(14)	6 330 (12)	23 323 (10)	−34 083 (30)	472 (10)	592 (10)	686 (12)	−68 (9)	24 (10)	−54 (10)
C(15)	7 402 (13)	16 529 (11)	−27 436 (34)	654 (12)	721 (12)	747 (13)	−41 (11)	8 (12)	29 (12)
C(16)	6 113 (14)	10 964 (11)	−40 490 (43)	680 (13)	602 (12)	1161 (19)	−63 (11)	21 (15)	−69 (15)
C(17)	3 610 (16)	12 169 (12)	−59 976 (46)	742 (15)	862 (16)	1117 (20)	−58 (13)	−92 (15)	−349 (17)
C(18)	2 496 (18)	18 885 (14)	−66 590 (38)	1025 (18)	1059 (19)	763 (16)	8 (17)	−170 (15)	−189 (15)
C(19)	3 931 (15)	24 474 (12)	−53 858 (34)	757 (15)	728 (12)	757 (15)	−36 (12)	−85 (12)	−3 (12)
N(1)	43 435 (10)	54 871 (8)	−6 866 (23)	550 (9)	668 (9)	530 (8)	−96 (8)	−16 (8)	111 (8)
N(2)	31 966 (10)	39 722 (8)	13 273 (20)	620 (9)	565 (8)	343 (7)	−71 (8)	9 (7)	−10 (6)
O(1)	55 228 (8)	58 242 (6)	−22 795 (19)	522 (7)	724 (8)	546 (7)	−92 (6)	13 (6)	103 (7)
O(2)	56 435 (9)	53 239 (10)	8 091 (23)	675 (9)	1508 (15)	641 (9)	−157 (10)	−119 (8)	351 (11)
O(3)	33 888 (10)	43 467 (7)	−18 308 (17)	1105 (12)	702 (9)	350 (6)	−168 (9)	28 (7)	3 (6)
O(4)	14 321 (9)	39 051 (7)	3 779 (21)	765 (9)	742 (8)	738 (9)	158 (8)	−78 (8)	−248 (8)
O(5)	16 554 (8)	29 101 (6)	−12 886 (19)	583 (7)	592 (7)	604 (7)	−16 (6)	−36 (6)	−135 (7)
				(b) *t*-B-G-P-OH					
C(1)	58 081 (68)	21 943 (12)	87 502 (34)	946 (40)	560 (21)	960 (32)	68 (26)	85 (34)	53 (23)
C(2)	56 917 (70)	22 525 (13)	63 646 (35)	906 (39)	893 (32)	986 (34)	82 (33)	−154 (34)	−505 (29)
C(3)	87 732 (57)	27 602 (16)	75 534 (39)	619 (27)	1384 (44)	914 (34)	−184 (29)	108 (29)	−354 (36)
C(4)	63 040 (52)	25 531 (11)	76 036 (30)	580 (20)	599 (18)	620 (20)	1 (16)	1 (19)	−151 (17)
C(5)	45 981 (56)	34 157 (10)	84 927 (26)	717 (22)	455 (16)	548 (17)	−50 (18)	−31 (19)	−8 (15)
C(6)	23 816 (61)	42 542 (10)	90 270 (26)	856 (29)	471 (17)	593 (20)	27 (20)	−126 (23)	−46 (16)
C(7)	6 045 (57)	46 061 (10)	83 228 (25)	782 (25)	455 (15)	488 (16)	−61 (18)	−45 (20)	27 (14)
C(8)	4 944 (61)	52 617 (11)	101 795 (25)	871 (33)	654 (20)	500 (19)	−51 (23)	−44 (23)	−106 (16)
C(9)	−5 068 (68)	58 489 (12)	102 224 (29)	1086 (36)	712 (24)	656 (24)	107 (27)	138 (28)	−183 (20)
C(10)	−25 803 (70)	58 263 (13)	93 470 (30)	973 (35)	881 (28)	670 (24)	272 (29)	202 (26)	45 (22)
C(11)	−18 242 (52)	54 331 (11)	82 814 (25)	644 (25)	576 (20)	506 (18)	21 (19)	44 (19)	107 (16)
C(12)	−6 421 (56)	57 507 (11)	72 093 (28)	706 (23)	520 (16)	587 (20)	54 (19)	105 (21)	66 (16)
N(1)	29 202 (51)	37 796 (9)	82 460 (22)	933 (25)	512 (15)	656 (18)	113 (17)	−244 (18)	−97 (14)
N((2)	−1 881 (42)	50 601 (8)	89 117 (18)	744 (18)	458 (12)	438 (12)	9 (15)	−6 (15)	35 (11)
O(1)	46 802 (35)	30 302 (7)	75 668 (19)	764 (14)	521 (10)	580 (12)	50 (12)	−104 (14)	−149 (10)
O(2)	58 592 (39)	34 287 (8)	94 257 (17)	877 (18)	684 (13)	566 (12)	130 (14)	−191 (14)	−185 (11)
O(3)	−528 (41)	44 724 (8)	72 394 (17)	1021 (18)	678 (12)	507 (12)	108 (14)	−182 (15)	−68 (11)
O(4)	13 970 (39)	58 506 (9)	71 645 (20)	706 (15)	1122 (20)	682 (16)	−173 (15)	16 (14)	278 (15)
O(5)	−21 493 (39)	59 154 (8)	63 347 (19)	720 (18)	889 (17)	732 (16)	71 (16)	36 (15)	380 (14)

[a] The temperature factor is of the form $\exp[-2\pi^2(h^2a^{*2}U_{11} + \ldots + 2klb^*c^*U_{23})]$.

and the collection of a second octant of data for the benzyl ester are reflected in smaller agreement indexes and a larger goodness of fit value (see Table I).

Calculations were carried out in Pasadena on an IBM 370-158 computer under the CRYM system of programs and in Bangalore on an IBM 360-44 under the GAASA[6] and Shiono[7] programs locally modified by B. Swaminatha Reddy and R. Ramani. Form factors for C, N, and O were from the International Tables[8] and for H from Stewart, Davidson, and Simpson.[9] No account was taken of anomalous dispersion.

Results and Discussion

Stereoscopic views[10] of the two molecules are shown in Figure 1, and drawings of the crystal structures are shown in Figures 2 and 3. Both molecules show an extended conformation, with no hint of the folded structure suggested by Deber[2a] for the free acid *t*-B-G-P-OH. Deber's suggestion of a folded structure was based on solid-state IR measurements which indicated that the absorption band associated with the C(5)-O(2) carbonyl grouping is at a lower frequency in several free acids such as *t*-B-G-P-OH when compared to benzyl derivatives such as *t*-B-G-P-OBz; he interpreted this frequency shift in terms of an intramolecular hydrogen bond O(5)-H···O(2) in the free acid, which would be sterically feasible for a folded conformation of the molecule. While we indeed find such a hydrogen bond [O(5)···O(2), 2.640 Å; H···O(2), 1.83 Å; O-H···O angle, 162.5°] in crystals of *t*-B-G-P-OH, it is *inter*molecular rather than intramolecular, relating the parent molecule at x,y,z to another at $\frac{1}{2} - x, 1 - y, -\frac{1}{2} + z$. This is the only hydrogen bond in *t*-B-G-P-OH, the other functional group N(1)-H not participating. In *t*-B-G-P-OBz the N(1)-H group is the only hydrogen bond donor available, and it forms a weak bond (N···O, 3.098 Å; H···O, 2.24 Å; N-H···O angle, 163.4°) to O(4) of the molecule at $\frac{1}{2} - x, 1 - y, -\frac{1}{2} + z$.

Various torsional angles are listed in Table IV. The only major conformational difference between the two molecules shows up in a relatively large twist about the N(1)-C(6) bond, and a much smaller twist about C(6)-C(7), in the benzyl ester; there is no reason to believe that these twists are due to other than packing forces. The torsion angles ϕ, C(7)-N(2)-C(11)-C(12), and ψ, N(2)-C(11)-C(12)-O(4), have values typical of prolyl peptides.[11] In both molecules the pyrrolidine ring is best described as an envelope with C(10), the flap, in

Table III. Coordinates ($\times 10^3$) and Isotropic Temperature Parameters of the Hydrogen Atoms[a]

	x	y	z	B	d(X-H), Å
			(a) t-B-G-P-OH		
H(1)	689 (7)	186 (2)	875 (4)	10.7 (1.2)	1.01
H(1′)	630 (7)	239 (2)	951 (3)	8.3 (1.1)	0.96
H(1″)	416 (7)	208 (2)	856 (4)	8.8 (1.2)	1.00
H(2)	648 (7)	190 (2)	636 (4)	9.8 (1.2)	0.95
H(2′)	589 (8)	252 (2)	565 (3)	10.1 (1.4)	0.99
H(2″)	405 (7)	214 (1)	622 (3)	7.6 (1.1)	0.99
H(3)	976 (7)	246 (1)	729 (4)	9.1 (1.1)	0.95
H(3′)	901 (7)	296 (1)	834 (3)	8.5 (1.1)	0.95
H(3″)	904 (8)	304 (2)	680 (4)	12.3 (1.4)	1.05
H(6)	369 (5)	448 (1)	920 (2)	4.8 (0.7)	0.94
H(6′)	179 (5)	415 (1)	992 (3)	6.0 (0.8)	1.02
H(8)	209 (6)	525 (1)	1030 (3)	6.4 (0.9)	0.92
H(8′)	−34 (6)	499 (1)	1085 (3)	8.7 (1.0)	1.06
H(9)	71 (6)	614 (1)	980 (3)	9.4 (1.1)	1.07
H(9′)	−89 (6)	597 (1)	1108 (3)	7.6 (0.9)	0.97
H(10)	−394 (7)	568 (2)	981 (4)	10.1 (1.2)	0.99
H(10′)	−319 (7)	618 (1)	897 (3)	8.9 (1.1)	1.00
H(11)	−306 (5)	524 (1)	790 (2)	4.8 (0.7)	0.94
H(N1)	203 (6)	378 (1)	755 (3)	6.2 (0.8)	0.89
H(O5)	−156 (8)	615 (2)	585 (4)	11.4 (1.3)	0.84
			(b) t-B-G-P-OBz		
H(1)	757 (2)	537 (1)	−280 (4)	8.2 (0.7)	0.98
H(1′)	688 (2)	501 (2)	−116 (5)	11.1 (0.8)	1.06
H(1″)	674 (2)	495 (2)	−349 (5)	9.9 (0.8)	0.94
H(2)	715 (2)	640 (2)	−492 (5)	11.9 (0.9)	1.05
H(2′)	618 (2)	677 (2)	−449 (4)	10.5 (0.8)	1.02
H(2″)	628 (2)	600 (1)	−546 (3)	8.9 (0.6)	0.90
H(3)	735 (2)	659 (1)	−122 (5)	9.9 (0.8)	0.94
H(3′)	674 (2)	623 (1)	36 (4)	10.4 (0.8)	0.92
H(3″)	642 (2)	687 (1)	−114 (4)	9.1 (0.7)	1.01
H(6)	336 (1)	539 (1)	139 (3)	6.5 (0.5)	1.02
H(6′)	422 (1)	502 (1)	182 (3)	4.7 (0.4)	0.88
H(8)	289 (1)	448 (1)	384 (4)	7.8 (0.6)	0.97
H(8′)	379 (1)	405 (1)	409 (3)	6.6 (0.5)	0.97
H(9)	208 (2)	350 (1)	435 (4)	9.1 (0.6)	1.02
H(9′)	300 (2)	323 (1)	558 (4)	7.7 (0.6)	1.02
H(10)	241 (1)	250 (1)	251 (3)	5.7 (0.5)	0.96
H(10′)	346 (1)	267 (1)	279 (3)	7.4 (0.5)	1.06
H(11)	313 (1)	311 (1)	−42 (3)	4.8 (0.4)	0.96
H(13)	66 (1)	341 (1)	−270 (4)	7.9 (0.6)	1.01
H(13′)	39 (1)	289 (1)	−86 (3)	6.8 (0.5)	0.97
H(15)	89 (1)	157 (1)	−128 (3)	5.1 (0.4)	1.01
H(16)	73 (2)	61 (1)	−353 (3)	8.1 (0.6)	1.01
H(17)	27 (2)	82 (1)	−701 (4)	10.4 (0.8)	1.02
H(18)	8 (2)	196 (1)	−801 (4)	10.3 (0.8)	0.95
H(19)	32 (2)	292 (1)	−580 (4)	8.4 (0.6)	0.95
H(N1)	409 (2)	557 (1)	−185 (3)	8.0 (0.6)	0.88

[a] Esd's in the bond lengths d(X-H) are in the range 0.03-0.05 Å.

an exo position relative to C(12); this is one of the two common conformations, and the torsion angles in the ring lie within the range found by Ashida and Kakudo[11] in a survey of prolyl-containing peptides. Details of the planarity of this and other groupings are given in Table V.

The conformation of the t-Boc group is closely similar in the two molecules; a drawing of the average, idealized conformation is shown in Figure 4. The C(4)-O(1) bond is cis to C(5)-O(2), rather than to C(5)-N(1), and the methyl groups are staggered with respect to O(2). The conformations about the C(4)-C(methyl) bonds lead to minimal repulsion between the hydrogen atoms, with no H···H distance below 2.4 Å; they also lead to relatively short H···O(2) distances, about 2.4 Å.

Bond distances and angles involving the heavier atoms are shown in Figure 5. For the most part, dimensions of the two molecules agree within the expected esd's of the differences, about 0.005-0.007 Å. Notable differences are centered around the carbamate grouping and can be correlated with the different hydrogen bonding patterns. In crystals of t-B-G-P-OH, where oxygen atom O(2) accepts a strong hydrogen bond, the C(5)-O(2) bond is appreciably longer and the neighboring C(5)-O(1) and C(5)-N(1) bonds are shorter than in crystals of the benzyl derivative, where no such hydrogen bond is formed. A second effect can be related to the N(1)-H···O hydrogen bond formed in the t-B-G-P-OBz crystals. In this bond the acceptor atom, O(4), lies 0.40 Å out of the plane of the donor grouping C(5)-N(1)-C(6); the donor hydrogen atom H(N1) is also displaced, by 0.27 Å, to the same side of this plane, resulting in considerable pyramidal character in the covalent bonding to N(1). The sp^3 character of the bonding to N(1) is also enhanced by the localization of double bond character in the C(5)-O(2) bond (vide supra), and both the

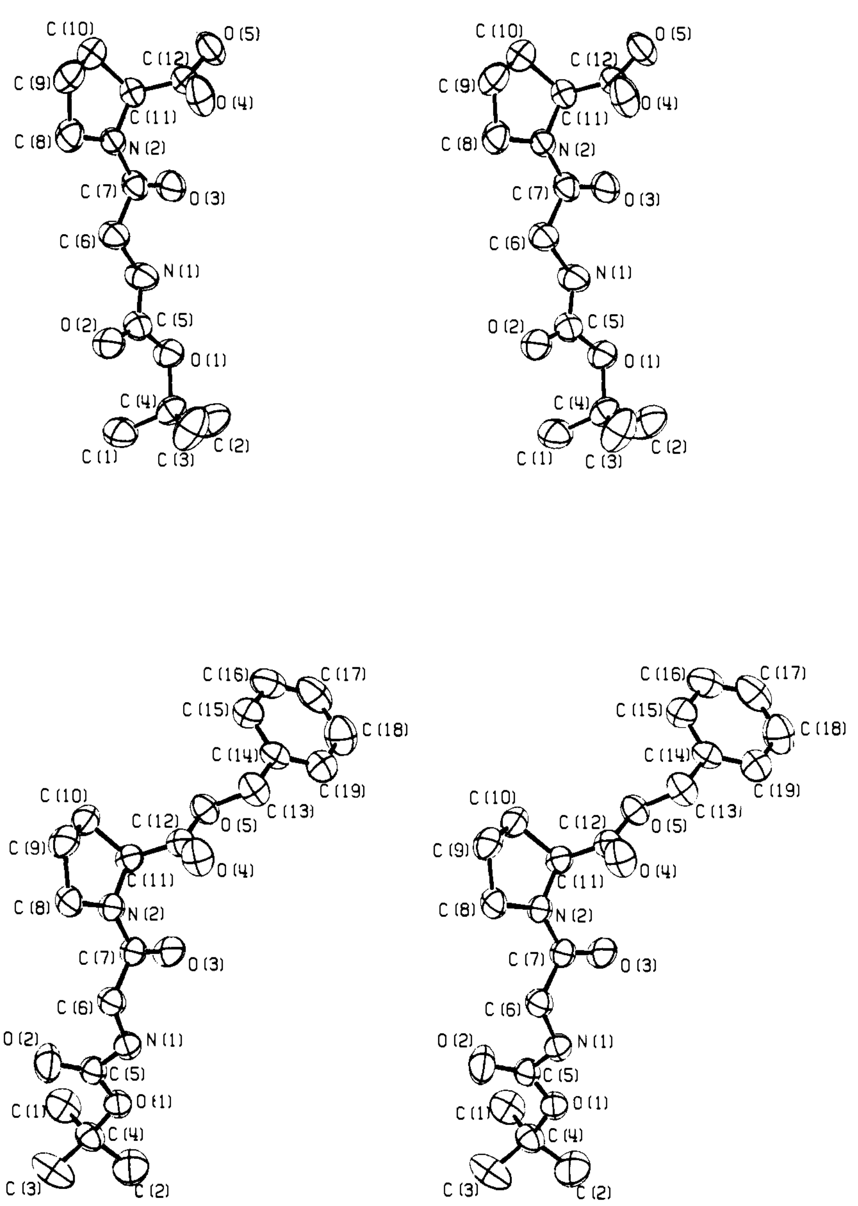

Figure 1. Stereoscopic drawings[10] of the molecules: top, *t*-B-G-P-OH; bottom, *t*-B-G-P·OBz. Temperature ellipsoids are drawn at the 50% probability level. Hydrogen atoms are omitted.

Table IV. Some Torsion Angles (Degrees)

	t-B-G-P-OH	*t*-B-G-P-OBz
C(1)-C(4)-O(1)-C(5)	61.8	63.4
C(2)-C(4)-O(1)-C(5)	179.9	−178.6
C(3)-C(4)-O(1)-C(5)	−63.7	−60.1
C(4)-O(1)-C(5)-O(2)	2.3	−1.4
O(1)-C(5)-N(1)-C(6)	−178.6	172.2
C(5)-N(1)-C(6)-C(7)	172.0	−109.5
N(1)-C(6)-C(7)-N(2)	177.8	164.4
C(6)-C(7)-N(2)-C(8)	0.6	2.7
C(6)-C(7)-N(2)-C(11)	177.4	−179.9
C(7)-N(2)-C(8)-C(9)	167.4	173.6
C(11)-N(2)-C(8)-C(9)	−9.7	−4.0
N(2)-C(8)-C(9)-C(10)	28.0	24.2
C(8)-C(9)-C(10)-C(11)	−35.9	−34.9
C(9)-C(10)-C(11)-N(2)	29.4	31.7
C(10)-C(11)-N(2)-C(8)	−12.3	−17.4
C(7)-N(2)-C(11)-C(12)	−70.5	−75.6
N(2)-C(11)-C(12)-O(4)	−25.7	−23.3
N(2)-C(11)-C(12)-O(5)	155.5	158.2

C(5)-N(1) and C(6)-N(1) distances are appreciably greater than in crystals of *t*-B-G-P-OH where the bonding about N(1) is closely planar (Table V).

The C-O distances in the three types of carbonyl groupings correlate well with the IR data of Deber,[2a] the highest frequency in the spectra, about 1740 cm^{-1}, being identified with the carboxylate grouping C(12)-O(4) which shows the shortest C-O distance and the lowest frequency, about 1645 cm^{-1}, with the amide grouping C(7)-O(3) which shows the longest distance. The change in frequency of the carbamate C(5)-O(2) band from about 1690 cm^{-1} in the benzyl ester to 1660 cm^{-1} in the hydrogen-bonded free acid is paralleled by the increase in C(5)-O(2) bond length. Thus, there is no reason to doubt that the crystals studied by us are identical with those studied by Deber.[2a]

In both compounds the bond angle O(1)-C(4)-C(2), at approximately 102°, is notably smaller than tetrahedral. Presumably this pinching is an accommodation to the overall crowding of the *t*-Boc group, aiding in the relief of the O(2)···C(1) and O(2)···C(3) contacts without worsening the methyl-methyl interactions. Further relief is afforded by the

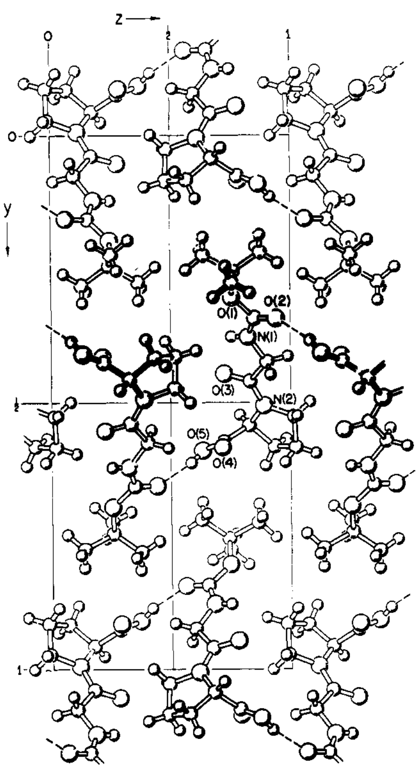

Figure 2. The crystal structure of *t*-B-G-P-OH, as viewed down the *a* axis. Dashed lines indicate hydrogen bonds.

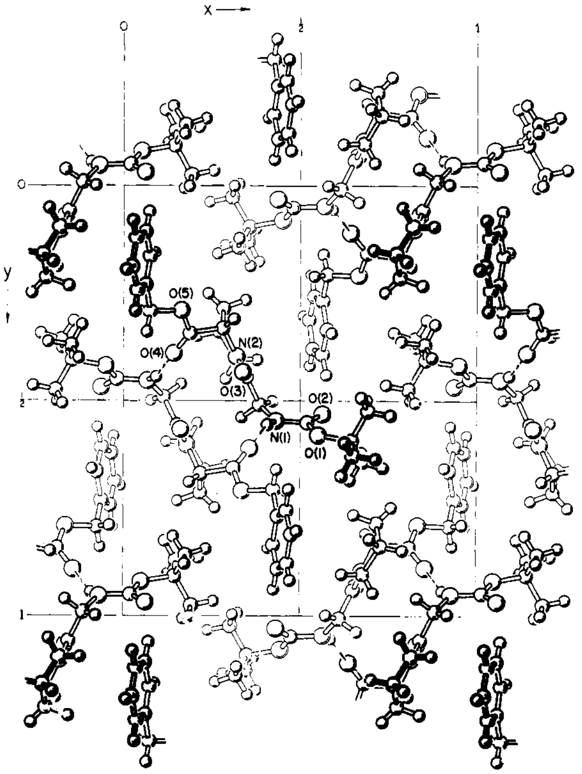

Figure 3. The crystal structure of *t*-B·G-P-OBz, as viewed up the *c* axis. Dashed lines indicate hydrogen bonds.

opening of the C(4)-O(1)-C(5) angle which, at an average value of 121.6°, is about 5° larger than usually found in ester groupings.

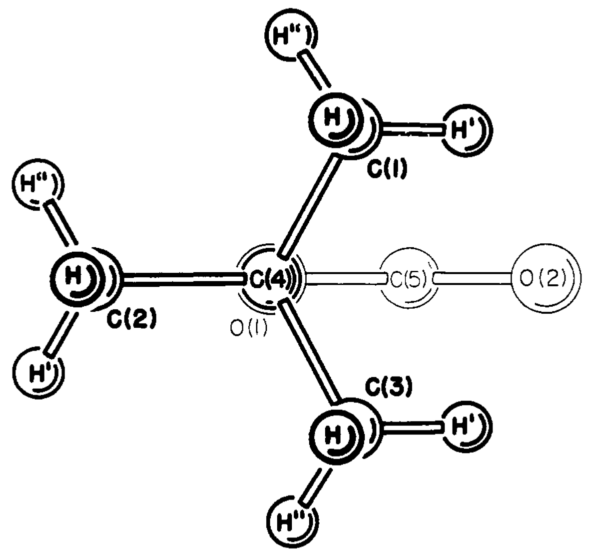

Figure 4. The idealized conformation of the *t*-Boc group. The molecular parameters of the two molecules, which are closely similar, have been averaged and the approximate mirror plane of symmetry has been made exact.

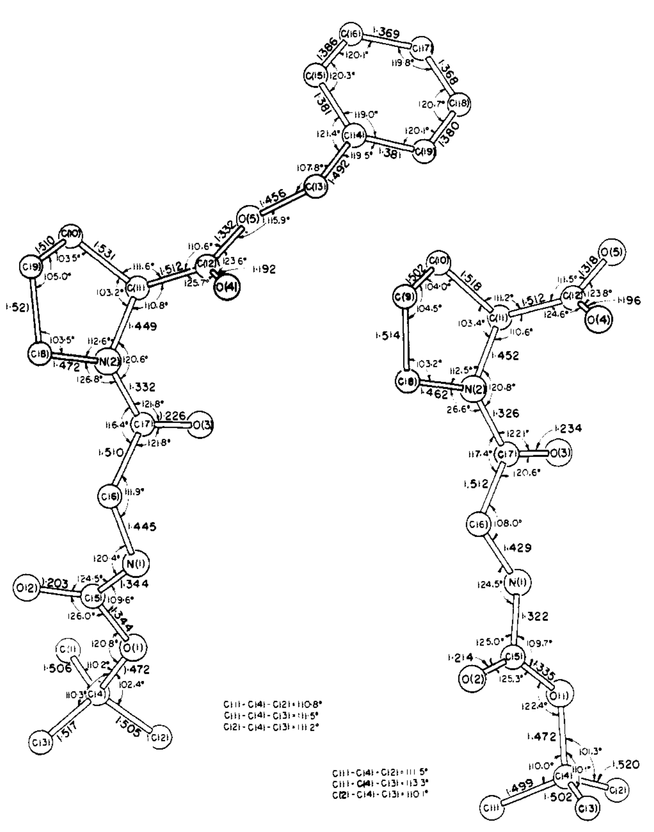

Figure 5. Bond distances and angles involving the C, N, and O atoms: left, *t*-B-G-P-OBz; right, *t*-B-G-P-OH.

Dimensions of the pyrrolidine ring, including the pronounced inequality of the bond angles at N(2), are in excellent agreement with values found for other prolyl peptides;[11] apparent small contractions of the C(9)-C(10) bond, as well as of the C(16)-C(17) and C(7)-C(18) bonds in the benzene ring of *t*-B-G-P-OBz, can be attributed to librational effects suggested by the relatively large thermal parameters of these atoms.

Table V. Some Planes of Interest[a]

	t-B-G-P-OH	*t*-B-G-P-OBz		*t*-B-G-P-OH	*t*-B-G-P-OBz
	Pyrrolidine Ring			*t*-Boc Group	
g_a	0.7934	−0.8808	g_a	0.6499	0.0712
g_b	0.3990	0.4683	g_b	0.5679	−0.9014
g_c	−0.4597	−0.0695	g_c	−0.5050	−0.4271
D, Å	0.398	−0.859	*D*, Å	1.863	−8.780
Atom	Dev, Å	Dev, Å	Atom	Dev, Å	Dev, Å
N(2)*	0.054	0.022	O(1)*	−0.001	−0.002
C(8)*	−0.051	−0.021	C(5)*	0.003	0.006
C(9)*	0.031	0.013	O(2)*	−0.001	−0.003
C(11)*	−0.034	−0.014	N(1)*	−0.001	−0.002
C(10)	−0.516	−0.529	C(4)	−0.060	−0.056
C(7)	0.266	0.133	C(6)	0.029	0.175
C(12)	1.322	1.364	H(N1)	0.03	0.16
	Peptide Group			Carboxyl Group	
g_a	0.7381	−0.9031	g_a	−0.1467	−0.3462
g_b	0.5417	0.4289	g_b	0.8550	−0.5175
g_c	−0.4021	0.0189	g_c	0.4974	0.7826
D, Å	2.716	−1.171	*D*, Å	15.531	−4.431
Atom	Dev, Å	Dev, Å	Atom	Dev, Å	Dev, Å
C(6)*	0.001	0.003	C(11)*	0.002	0.002
C(7)*	−0.003	−0.010	C(12)*	−0.006	−0.008
O(3)*	0.001	0.004	O(4)*	0.002	0.003
N(2)*	0.001	0.003	O(5)*	0.002	0.002
N(1)	−0.056	−0.373	N(2)	−0.569	−0.510
C(8)	0.019	0.083	C(10)	1.421	1.428
C(11)	0.054	−0.009	C(13)		0.057
H(N1)	−0.15		H(O5)	0.18	

[a] Least-squares planes were passed through the atoms indicated with an asterisk; the direction cosines *g* are relative to the crystallographic axes; and *D* is the origin to plane distance.

Conclusion

Our results indicate that the differences in the IR spectra of these two crystals, which were interpreted by Deber[2a] as suggesting an intramolecular hydrogen bond and hence a folded conformation for the free acid, are actually due to an intermolecular hydrogen bond. They do not, of course, rule out the possibility that similar frequency shifts found by Deber for other crystalline peptides might, in some cases at least, be indicative of a folded conformation. In all of the peptides studied by Deber, the frequency shifts are associated specifically with the C=O grouping which would be expected to accept a hydrogen bond if the molecule forms a β turn, and at first glance it seems rather surprising that the packing forces in the various crystal structures would so consistently lead to the same C=O group being the acceptor of an intermolecular hydrogen bond. Clearly, this point warrants further study.

Preliminary results of some of this work were recently presented and discussed without our prior knowledge and without proper acknowledgment.[12]

References and Notes

(1) (a) This investigation was supported, in part, by the CNR (Italy) through a NATO fellowship to E.B., in part by the U.S. Public Health Service through Grant No. GM 16966 from the National Institute of General Medical Sciences, National Institutes of Health, to R.E.M., and, in part, by the CSIR, India. (b) California Institute of Technology. (c) Indian Institute of Science.

(2) (a) C. M. Deber, *Macromolecules*, **7**, 47 (1974); (b) C. M. Venkatachalam, *Biopolymers*, **6**, 1425 (1968), and references cited therein.

(3) G. Germain, P. Main, and M. M. Woolfson, *Acta Crystallogr., Sect. A*, **27**, 368 (1971).

(4) A. J. C. Wilson, *Nature (London)*, **150**, 151 (1942).

(5) J. Karle and H. Hauptman, *Acta Crystallogr.*, **9**, 635 (1956).

(6) O. Lindgren, O. Lindquist, and J. Nyburg, *Acta Chem. Scand.*, **24**, 732 (1970).

(7) R. Shiono, private communication (1968).

(8) "International Tables for X-Ray Crystallography", Vol. III, Kynoch Press, Birmingham, 1962, pp 202–203.

(9) R. F. Stewart, E. R. Davidson, and W. T. Simpson, *J. Chem. Phys.*, **42**, 3175 (1965).

(10) C. K. Johnson, "ORTEP, a Fortran Thermal Ellipsoid Program for Crystal Structure Illustrations", U.S. Atomic Energy Commission Report ORNL-3794, Oak Ridge National Laboratory, Oak Ridge, Tenn., 1965.

(11) T. Ashida and M. Kakudo, *Bull. Chem. Soc. Jpn.*, **47**, 1129 (1974).

(12) E. Benedetti, M. Palumbo, G. M. Bonora, and C. Toniolo, *Macromolecules*, **9**, 417 (1976).