Crystal Structures and Conformational Calculations of Fragments of Alamethicin Containing Aminoisobutyric Acid[1]

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Abstract: The crystal structures of four peptide fragments (Boc-Pro-Aib-Ala-Aib-OBzl, Boc-Leu-Aib-Pro-OBzl, Boc-Leu-Aib-Pro-OH, and Boc-Gly-Aib-OH) of alamethicin containing five aminoisobutyric acid (Aib) residues have been determined. In all cases, the conformation of the Aib residue is in excellent agreement with the predictions of previous theoretical calculations. Viewed together with other observations in the literature, these data clearly demonstrate that the crystallographically determined average value of ±(50, 42) for (ϕ, ψ) for Aib is a low-energy form. Systematic calculations of the possible conformers for acetyl-Aib-Ala-Aib-methylamide show four minima with one corresponding closely to that observed in Boc-Pro-Aib-Ala-Aib-OBzl.

Alamethicin, a peptide antibiotic produced by the fungus *Trichoderma viride*, has been the subject of considerable study due to the unusual electrical properties it confers when added to artificial bilayers.[2] One difficulty has been the characterization of the structure of this molecule, which was originally postulated to by cyclic,[3] then linear with blocked termini,[4] and finally revised with regard to peptide linkage.[5] This final structure has recently been shown to give a material identical with the major component of alamethicin upon synthesis.[6]

During the syntheses of the proposed structures for alamethicin,[6] a number of crystalline fragments were prepared, and the structures of several of these are the subject of this paper. The presence within two of these peptides, residues 2–5 and 12–14, of four residues of aminoisobutyric acid (Aib) offers the first opportunity to check predictions made on the basis of theoretical calculations by Marshall and Bosshard[7] in 1972 and subsequently confirmed by two other groups.[8] A preliminary publication of the crystal structure of residues 1–4 of alamethicin,[9] the determination of the structure of tosyl-$(Aib)_5$-OMe,[10] and the determination of the structure of (*Z*)-Aib-Pro-$NHCH_3$[11] offers further confirmation of these predictions. These observations provide a firm experimental foundation for the incorporation of amino acids in which the α-proton has been replaced with a methyl group as a means of restricting the backbone conformation of the peptide at that residue[12] as well as a means of inhibiting peptide degradation.[13] In addition, the constraints introduced by the aminoisobutyric residues may be sufficiently strong that the crystal structures observed in fragments of alamethicin may relate directly to the structure of the intact molecule.

Experimental Section

Single crystals of Boc-Gly-Aib-OH, Boc-Leu-Aib-Pro-OH, and Boc-Pro-Aib-Ala-Aib-OBzl were grown from an ethyl acetate/petroleum ether solution while crystals of Boc-Leu-Aib-Pro-OBzl were grown from an acetone/ethyl acetate solution saturated with water. No attempt was made to exclude either air or moisture during crystallization of all four samples. Crystallographic diffraction data for the dipeptide and both tripeptides were measured on an Enraf-Nonius CAD-4 diffractometer; data for the tetrapeptide were measured on a Picker diffractometer. The radiation employed for all four crystals was Ni-filtered Cu Kα radiation. Pertinent unit cell data are given in Table I. Between two and five standard reflections were measured during the course of the data collection for each of the four crystals. A 15% variation for the two standards of the dipeptide was the greatest observed for a particular set of standard reflections, while the smallest variation, observed for the five standards of the Boc-Leu-Aib-Pro-OBzl crystal, consisted of a decrease of 5% during the first 3 h of data collection followed by a variation of less than 3% for the remainder of the time. Intensities for all four samples were corrected for Lorentz and polarization (Lp) factors but not for extinction or absorption. Real and imaginary dispersion corrections were applied to the atomic scattering factors.[14] Data considered unobserved on the basis of a $2\sigma(I)$ test were assigned a zero weight and not included in the refinement. Variances for each F for the dipeptide and both tripeptides were calculated according to the method of Stout and Jensen [$\sigma^2(F) = k/4(L_p)I[\sigma^2(I) + (0.06I)^2]$; $w(F) = 1/\sigma^2(F)$].[15] Weights for the tetrapeptide were calculated on the basis of a modified Hughes weighting scheme ($w = a/|F_o|$, $|F_o| > a$; $w = |F_o|/a$, $|F_o| < a$; $a = 15$).[16]

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Table I. Crystal Data

	Boc-Gly-Aib-OH	Boc-Leu-Aib-Pro-OH	Boc-Leu-Aib-Pro-OBzl	Boc-Pro-Aib-Ala-Aib-OBzl
formula	$C_{11}H_{20}O_5N_2$	$C_{20}H_{35}N_3O_6 \cdot H_2O$	$C_{27}H_{41}N_3O_6$	$C_{28}H_{42}N_5O_7$
formula wt	260.29	431.53	503.64	546.67
a, Å	13.261 (1)	10.853 (4)	22.572 (3)	11.147 (1)
b, Å	9.302 (1)	23.646 (5)	10.153 (1)	13.877 (7)
c, Å	11.461 (1)	9.748 (1)	6.134 (1)	10.417 (1)
α, deg	90	90	90	90
β, deg	102.57 (1)	90	89.98 (1)	100.9 (1)
γ, deg	90	90	90	90
space group	$P2_1/c$	$P2_12_12_1$	$P2_1$	$P2_1$
Z	4	4	2	2
V, Å^3	1379.87	2501.63	1405.76	1582.30
ρ_{calcd}, g cm^{-3}	1.253	1.146	1.190	1.147
λ(Cu Kα), cm^{-1}	8.402	7.24	8.40	7.26
$(\sin\theta_{max})/\lambda$, Å^{-1}	0.61	0.53	0.63	0.58
tot indpt data	2834	1822	3052	2767
obsd data	2466	1017	2628	2478
R^a	0.042 (0.070 all data)	0.093 (0.160 all data)	0.046 (0.056 all data)	0.087 (0.096 all data)
$R_w{}^b$	0.065	0.054	0.058	0.058
S	2.095	3.371	1.972	0.364

[a] $R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$. [b] $R_w = [\Sigma w(|F_o| - |F|)^2/\Sigma w|F_o|^2]^{1/2}$. [c] $S = \Sigma w\Delta^2/m - n$; m = number observations; n = number of parameters.

Table II. Positional Parameters of the Nonhydrogen Atoms of Boc-Gly-Aib-OH[a]

atom	x/a	y/b	z/c
C(1)	0.52849 (9)	0.2589 (1)	0.3665 (1)
C(1A)	0.6025 (1)	0.2638 (2)	0.2819 (1)
C(2)	0.36289 (9)	0.3695 (1)	0.4658 (1)
C(2A)	0.35076 (9)	0.2314 (1)	0.3902 (1)
C(2B1)	0.2451 (1)	0.2403 (2)	0.3032 (1)
C(2B2)	0.3585 (1)	0.0992 (2)	0.4703 (2)
C(3)	0.77605 (9)	0.2489 (1)	0.3997 (1)
C(4)	0.9250 (1)	0.2674 (2)	0.5670 (1)
C(5)	1.0077 (1)	0.2075 (2)	0.5076 (2)
C(6)	0.9650 (1)	0.3945 (2)	0.6467 (2)
C(7)	0.8808 (2)	0.1537 (2)	0.6367 (1)
N(1)	0.69853 (8)	0.3322 (1)	0.3372 (9)
N(2)	0.42993 (8)	0.2291 (1)	0.31868 (8)
O(1)	0.55831 (7)	0.2824 (1)	0.47462 (7)
O(3)	0.78504 (9)	0.1212 (1)	0.3856 (1)
O(4)	0.84278 (7)	0.3325 (1)	0.47563 (8)
O(2′)	0.35453 (8)	0.3734 (1)	0.56867 (7)
O(2″)	0.37721 (8)	0.4838 (1)	0.40394 (7)

[a] In this and the following tables, the estimated standard deviations are given in parentheses.

Structure Determination and Refinement

Boc-Gly-Aib-OH. This structure was solved with use of the direct-methods program MULTAN.[17] The correct solution was easily recognizable on the basis of the residual, and an E map based upon the phases of the 255 largest E's revealed the positions of all nonhydrogen atoms. After several cycles of full-matrix least-squares refinement, all hydrogen atoms were located. The refinement was continued, treating the vibration of nonhydrogen atoms anisotropically and the vibration of hydrogen atoms isotropically. At this point, an examination of the calculated and observed structure factors revealed that 17 reflections were in very poor agreement and had apparently been incorrectly measured. These data were then assigned weights of zero and were excluded from the subsequent two cycles of least-squares refinement which was halted when the largest shift divided by its standard deviation was less than 0.3. Residuals are listed in Table I. The largest peak of a final difference Fourier map, for which a single hydrogen atom had been excluded from the structure factor calculations, corresponded to the missing hydrogen atom; other smaller peaks were more than 0.40 Å distant from any atom in the unit cell. Positional parameters for nonhydrogen atoms are given in Table II.

Boc-Leu-Aib-Pro-OH. This structure was solved in a routine fashion with use of the direct-methods program QTAN.[18] The solution was identified correctly by both the residual and the negative quartet figure of merit.[19] The resulting E map revealed the positions of 25 of the 29 nonhydrogen atoms. Fourier refinement provided coordinates for the remaining atoms of the peptide as well as for the water molecule. The structure was refined by full-matrix least squares, treating the vibration of all atoms isotropically (paucity of observed data prohibited anisotropic refinement). Hydrogen atom contributions were included in the final three cycles of least squares by calculating their positions on the basis of the heavy-atom positions at the end of each cycle; methyl groups were assumed to have a staggered configuration. No contribution was included for the hydrogen of the carboxyl group or of the water molecule. After the refinement converged at a residual of 0.11, bond distances and angles in several cases deviated significantly from that expected for a peptide. A $\delta(R)$ plot[20] was then constructed which showed that 35 data points had apparently been measured incorrectly and did not follow the expected

Table III. Positional Parameters of the Nonhydrogen Atoms of Boc-Leu-Aib-Pro-OH

atom	x/a	y/b	z/c
C(1)	0.6191 (8)	0.8170 (3)	1.194 (1)
C(1A)	0.7093 (8)	0.8506 (3)	1.291 (1)
C(1B)	0.7805 (8)	0.8085 (3)	1.384 (1)
C(1D1)	0.946 (1)	0.7939 (5)	1.559 (1)
C(1D2)	0.969 (1)	0.8725 (5)	1.386 (1)
C(1G)	0.8815 (9)	0.8394 (4)	1.474 (1)
C(2)	0.5122 (8)	0.7937 (3)	0.9158 (9)
C(2A)	0.6148 (8)	0.7584 (4)	0.980 (1)
C(2B1)	0.7084 (9)	0.7464 (4)	0.864 (1)
C(2B2)	0.5684 (9)	0.7044 (4)	1.043 (1)
C(3)	0.4128 (9)	0.8580 (4)	0.670 (1)
C(3A)	0.4201 (9)	0.8767 (4)	0.821 (1)
C(3B)	0.450 (1)	0.9386 (4)	0.834 (1)
C(3D)	0.6308 (9)	0.8847 (4)	0.909 (1)
C(3G)	0.587 (1)	0.9372 (4)	0.826 (1)
C(4)	0.6039 (9)	0.9389 (4)	1.319 (1)
C(5)	0.4738 (9)	1.0196 (4)	1.372 (1)
C(6)	0.405 (1)	1.0189 (5)	1.231 (1)
C(7)	0.3830 (9)	1.0307 (4)	1.482 (1)
C(8)	0.570 (1)	1.0636 (4)	1.364 (1)
N(1)	0.6359 (6)	0.8884 (3)	1.3694 (8)
N(2)	0.6847 (7)	0.7909 (3)	1.0871 (7)
N(3)	0.5231 (6)	0.8471 (3)	0.8903 (7)
O(1)	0.5095 (6)	0.8188 (2)	1.1988 (6)
O(2)	0.4163 (5)	0.7667 (2)	0.8831 (6)
O(4)	0.6475 (6)	0.9585 (3)	1.2161 (8)
O(5)	0.5206 (6)	0.9630 (2)	1.4044 (6)
O(3′)	0.5021 (6)	0.8406 (2)	0.6089 (7)
O(3″)	0.3056 (6)	0.8688 (3)	0.6155 (7)
O(1W)	0.8196 (6)	0.6368 (2)	0.6597 (7)

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Table IV. Positional Parameters of the Nonhydrogen Atoms of Boc-Leu-Aib-Pro-OBzl

atom	*x/a*	*y/b*	*z/c*
C(1)	0.81649 (9)	0.6782 (2)	0.4071 (3)
C(1A)	0.83914 (9)	0.5927 (2)	0.5954 (3)
C(1B)	0.7890 (1)	0.5131 (3)	0.6981 (4)
C(1D)	0.7573 (2)	0.3486 (4)	0.9799 (6)
C(1D2)	0.8358 (2)	0.2876 (4)	0.7093 (7)
C(1G)	0.8089 (1)	0.3982 (3)	0.8399 (4)
C(2)	0.7385 (1)	0.8405 (3)	0.1080 (4)
C(2A)	0.7757 (1)	0.8935 (3)	0.3007 (4)
C(2B1)	0.7372 (1)	0.9984 (3)	0.4136 (5)
C(2B2)	0.8328 (1)	0.9556 (3)	0.2140 (5)
C(3)	0.6133 (1)	0.8502 (4)	−0.0878 (4)
C(3A)	0.6517 (1)	0.7329 (4)	−0.0373 (4)
C(3B)	0.6145 (2)	0.6169 (4)	0.0468 (7)
C(3D)	0.6731 (1)	0.6918 (4)	0.3419 (5)
C(3G)	0.6115 (2)	0.6402 (5)	0.2875 (7)
C(4)	0.91804 (9)	0.7379 (3)	0.7086 (3)
C(5)	0.9924 (1)	0.8865 (3)	0.8656 (4)
C(6)	0.9736 (2)	1.0043 (4)	0.7324 (6)
C(7)	1.0038 (1)	0.9241 (4)	1.1023 (5)
C(8)	1.0454 (1)	0.8150 (4)	0.7706 (6)
C(9)	0.5490 (2)	0.9349 (5)	−0.3691 (6)
C(10)	0.4910 (1)	0.8683 (4)	−0.4154 (5)
C(11)	0.4610 (2)	0.7938 (5)	−0.2613 (6)
C(12)	0.4072 (2)	0.7405 (5)	−0.3053 (7)
C(13)	0.3817 (2)	0.7558 (5)	−0.5080 (7)
C(14)	0.4117 (2)	0.8250 (5)	−0.6652 (6)
C(15)	0.4658 (1)	0.8803 (4)	−0.6200 (5)
N(1)	0.86852 (8)	0.6705 (2)	0.7616 (3)
N(2)	0.79050 (8)	0.7926 (2)	0.4604 (3)
N(3)	0.69165 (9)	0.7615 (3)	0.1446 (3)
O(1)	0.81900 (8)	0.6379 (-)	0.2175 (3)
O(2)	0.7478 (1)	0.8812 (3)	−0.0779 (3)
O(4)	0.93659 (8)	0.7464 (2)	0.5235 (3)
O(5)	0.94200 (7)	0.7938 (2)	0.8862 (2)
O(3'')	0.5909 (1)	0.8369 (3)	−0.2881 (4)
O(3')	0.6013 (1)	0.9377 (3)	0.0326 (4)

distribution of errors. These data were then excluded from the refinement which was continued in the same manner as described above. When the refinement had converged at the residual listed in Table I, the results $\delta(R)$ plot[20] was linear with a slope of 3.14 and an intercept of 0.19, indicating that the errors in the data are normally distributed and furthermore that the standard deviations are underestimated by a factor of approximately 3.1. A final difference map was then calculated in which an α-carbon hydrogen atom was omitted from the structure factor calculation. The four largest peaks of this map were approximately 0.8 Å from four different oxygen atoms and most likely result from thermal motion which is uncorrected by the isotropic approximation. The sixth largest peak, 68% of the height of the largest, corresponded to the missing hydrogen atom. The relative ordering of these peaks is not surprising since hydrogen atom positions were calculated rather than observed, a result of the limited amount of data. Positional parameters are given in Table III.

Boc-Leu-Aib-Pro-OBzl. This structure was solved with use of the direct-methods program QTAN.[18] The correct solution was easily identified from the residual (0.25) and negative quartet figure of merit (−0.38).[19] Coordinates of 26 atoms were obtained from the initial *E* map. Several cycles of Fourier refinement revealed the positions of the remaining atoms. Nearly all hydrogen atom positions were located following several cycles of full-matrix least-squares refinement that treated the vibration of the nonhydrogen atoms anisotropically. Hydrogen atom positional and isotropic thermal parameters were refined in subsequent cycles of least squares. It was not possible to unambiguously locate all three hydrogen atoms of one of the terminal methyl groups of the leucine side chain nor both hydrogens of the benzyl methylene carbon atom. As a result, the positions of all five of these hydrogen atoms were calculated from geometrical considerations at the end of each cycle of least squares. When the refinement had converged, a $\delta(R)$ plot[20] was constructed which was linear with the exception of 14 points, suggesting that the distribution of errors for these data are different from that of the remainder of the data. Therefore, these 14 data were excluded from the subsequent two cycles of least squares. Following convergence at the residuals listed in Table I, a second $\delta(R)$ plot[20] was constructed which was linear with a slope of 1.79 and an intercept of 0.07, again suggesting that the standard deviations are underestimated by a factor of about 1.8 and that there are no significant differences between F_c and F_o. A final difference map was then calculated in which an α-carbon hydrogen atom was omitted from the structure-factor calculation. The largest peak of this map corresponded to the omitted hydrogen atom. The second largest peak, 40% of that of the first, was more than 0.5 Å distant from any other atom. Positional parameters of the nonhydrogen atoms are listed in Table IV.

Table V. Positional Parameters of the Nonhydrogen Atoms of Boc-Pro-Aib-Ala-Aib-OBzl

atom	*x/a*	*y/b*	*z/c*
C(1)	−0.3879 (4)	0.2074 (4)	0.7145 (4)
C(1A)	−0.3310 (5)	0.1127 (4)	0.7480 (5)
C(1B)	−0.4003 (5)	0.0502 (5)	0.8364 (6)
C(1C)	−0.2997 (6)	−0.0165 (6)	0.9039 (6)
C(10)	−0.1833 (5)	0.0412 (5)	0.9279 (5)
C(2)	−0.4000 (5)	0.4193 (4)	0.6583 (5)
C(2A)	−0.4164 (5)	0.3734 (5)	0.7922 (4)
C(2B1)	−0.5571 (5)	0.3670 (5)	0.7938 (5)
C(2B2)	−0.3526 (6)	0.4361 (5)	0.9000 (6)
C(3)	−0.2863 (5)	0.3898 (5)	0.3868 (5)
C(3A)	−0.2550 (6)	0.4469 (5)	0.5141 (5)
C(3B)	−0.1164 (6)	0.4650 (5)	0.5467 (6)
C(4)	−0.2458 (5)	0.2462 (5)	0.2013 (4)
C(4A)	−0.3445 (5)	0.2355 (5)	0.2837 (4)
C(4B2)	−0.4674 (6)	0.2508 (9)	0.2007 (6)
C(4B1)	−0.3319 (7)	0.1320 (6)	0.3353 (6)
C(5)	−0.1217 (5)	0.1656 (4)	0.7794 (5)
C(6)	0.0983 (5)	0.2035 (5)	0.8269 (6)
C(7)	0.1178 (6)	0.1836 (5)	0.6906 (6)
C(8)	0.2018 (6)	0.1613 (7)	0.9305 (8)
C(9)	0.0860 (6)	0.3090 (6)	0.8556 (6)
C(10)	−0.0369 (5)	0.2634 (9)	0.1952 (5)
C(11)	0.0708 (5)	0.2927 (4)	0.2779 (5)
C(12)	0.1695 (6)	0.2306 (7)	0.3162 (7)
C(13)	0.2789 (7)	0.2666 (7)	0.4032 (7)
C(14)	0.2711 (7)	0.3518 (6)	0.4339 (7)
C(15)	0.1911 (8)	0.4124 (8)	0.4049 (9)
C(16)	0.0871 (7)	0.3839 (6)	0.3250 (7)
N(1)	−0.2068 (4)	0.1140	0.8236 (4)
N(2)	−0.3628 (4)	0.2758 (3)	0.8072 (4)
N(3)	−0.2880 (4)	0.4008 (3)	0.6279 (3)
N(4)	−0.3201 (4)	0.2992 (3)	0.3963 (3)
O(1)	−0.4565 (3)	0.2229 (3)	0.6092 (3)
O(2)	−0.4734 (4)	0.4758 (3)	0.6010 (4)
O(3)	−0.2804 (4)	0.4276 (3)	0.2821 (3)
O(5)	−0.1414 (3)	0.2188 (3)	0.6861 (3)
O(6)	−0.0090 (3)	0.1512 (3)	0.8559 (3)
O(4'')	−0.1351 (3)	0.2517 (4)	0.2710 (3)
O(4')	−0.2666 (4)	0.2396 (4)	0.0833 (3)

Boc-Pro-Aib-Ala-Aib-OBzl. A sustained attempt over the period of several years was made to solve this structure through the use of MULTAN.[17] The use of QTAN[18] in the manner described for the two previous structures was also unsuccessful. In both bases, the figures of merit suggested that the phases were incorrect, and the *E* maps which were calculated revealed no recognizable fragments. The cosines of the triple invariants were then estimated on the basis of the ten-magnitude second neighborhood.[21] From these estimates, 242 invariants were found to be unreliable and were therefore eliminated from the convergence maps. The starting phase set for QTAN consisted of eight twofold ambiguities along with the phases of 35 restricted *E*'s which were obtained from the most reliably estimated restricted phase triple invariants. The correction solution had a residual of 0.39 and a negative quartet figure of merit[19] of −0.24; the residual by itself was not sufficient to identify the correct solution. The resulting *E* map revealed the positions of 23 nonhydrogen atoms, and several cycles of Fourier refinement were sufficient to locate all the remaining nonhydrogen atoms. Some difficulty was encountered in locating atoms of the benzyl group, most likely a result of high thermal motion. The structure was refined, treating the vibration of the benzyl group isotropically and the vibration of the remaining nonhydrogen atoms anisotropically by full-matrix least squares. Hydrogen atom contributions to the structure factors were included in the latter stages of refinement by calculating their positions on the basis of idealized geometry. The refinement converged at the residuals listed in Table I; positional parameters are listed in Table V.

Theoretical Calculations. Computations were carried out for acetyl-Aib-L-Ala-Aib-methylamide with the software, BURLESK,[22] a systematic

(21) G. Kruger, E. A. Green, and H. A. Hauptman, private communication, 1977.

Table VI. Summary of BURLESK Calculations for Acetyl-Aib-Ala-Aib-methylamide

run		range of torsional values. Aib-1[a]	L-Ala[1]	Aib-2[a]	global E min, kcal/mol[b]
1	ϕ	−67.5 to −33.75	−180 to +180	33.75 to 67.5	−4.2
	ψ	−67.5 to −22.5	−180 to +180	22.5 to 67.5	
2	ϕ	33.75 to 67.5	−180 to +180	−67.5 to −33.75	−4.0
	ψ	22.5 to 67.5	−180 to +180	−67.5 to −22.5	
3	ϕ	−67.5 to −33.75	−180 to +180	−67.5 to −33.75	−4.6
	ψ	−67.5 to −22.5	−180 to + 180	−67.5 to −22.5	
4	ϕ	33.75 to 67.5	−180 to +180	33.75 to 67.5	−4.4
	ψ	22.5 to 67.5	−180 to +180	22.5 to 67.5	

[a] Torsional angle increment: $2\pi/32 = 11.25°$. [b] Scale of Kitaygorodsky potential energy function[23] with K2 parameterization of Venkatachalam and Ramachandran.[24]

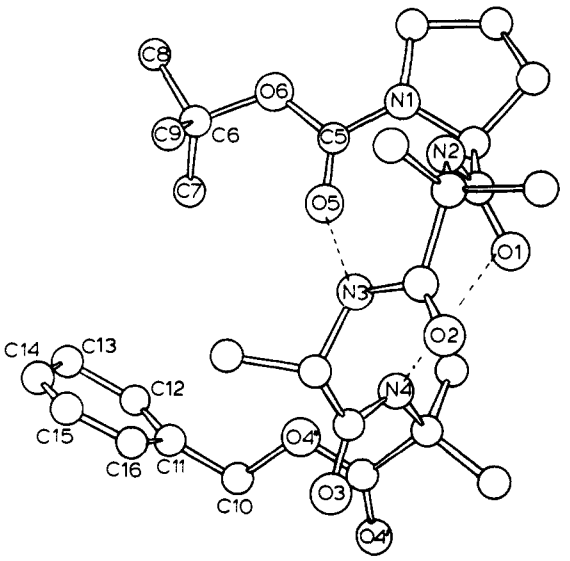

Figure 1. Observed conformation of Boc-Pro-Aib-Ala-Aib-OBzl.

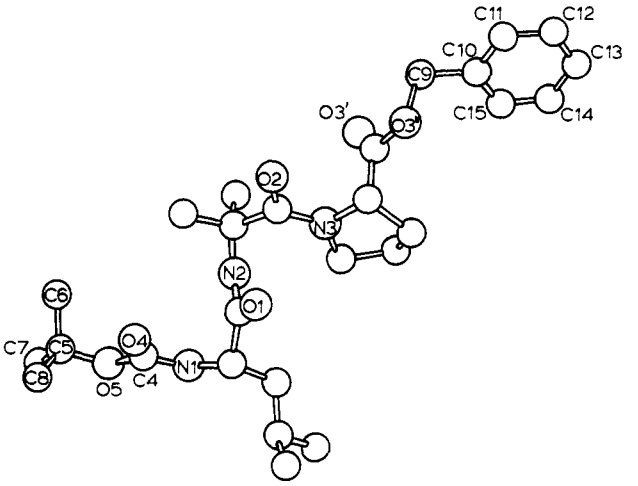

Figure 2. Observed conformation of Boc-Leu-Aib-Pro-OBzl.

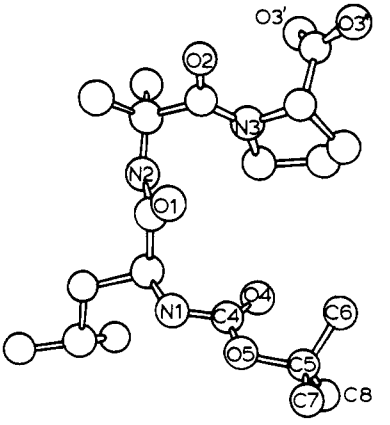

Figure 3. Observed conformation of Boc-Leu-Aib-Pro-OH.

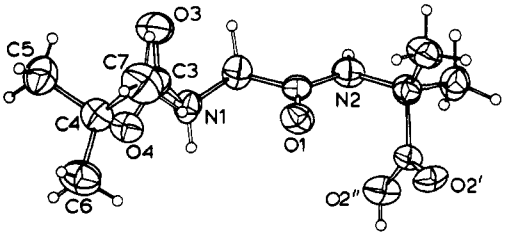

Figure 4. Observed conformation of Boc-Gly-Aib-OH.

conformational space search which rotates preassigned rotatable bonds in a combinatorial manner by incrementally increasing torsion angles within specified ranges. The particular version of BURLESK used was written in assembly language for a PDP-12 minicomputer with 8K of 12-bit/word memory, augmented by a hardware floating-point processor (FPP-12, Digital Equipment Corp.) for potential energy calculations. The Kitaygorodsky[23] potential energy function was evaluated for each conformation with the K2 constants for atomic interactions as published by Venkatachalam and Ramachandran.[24] Methyl groups are treated as single, bulky atoms in the parameterization, which eliminates the need for their individual rotations and reduces the problem to one of 6 degrees of freedom (rotatable bonds).

Since prior calculations had shown[7] that the Aib residues will prefer conformations close to either a right- or left-handed helix, the data were composed from four separate BURLESK runs as summarized in Table VI. These represent an exploration of all values for ϕ and ψ of the L-Ala residue with the four possible combinations of helical *regions* for the two Aib residues, Aib-1 and Aib-3.

Results

The observed conformations of the tetrapeptide, both tripeptides, and the dipeptide are illustrated in Figures 1–4, respectively. Bond distances and bond angles for each peptide are listed in Tables VII and VIII. In nearly all cases, bond distances and angles are within three standard deviations from that expected for a peptide. One notable exception is the distances and angles of the benzyl group of the tetrapeptide. Larger thermal parameters and the difficulty of initially locating all the atoms of the ring suggest a considerable amount of thermal motion or even disorder of these atoms which may account for the poor geometry. All peptide linkages are trans; torsion angles for all four peptides are listed in Table IX.

The conformation of the first three residues of the tetrapeptide is best described as approximating a single turn of a 3_{10}-helix. This helix is stabilized by two intramolecular hydrogen bonds; the first arises as a result of the amino group of Ala[3] donating a proton to the carbonyl oxygen of Boc (N–O distance 3.01 Å) while a

(22) H. E. Bosshard, C. D. Barry, J. M. Fritsch, R. A. Ellis, and G. R. Marshall, *Proc. Summer Computer Simul. Conf.*, *1*, 581 (1972).
(23) A. I. Kitaygorodsky, *Tetrahedron*, 14 230 (1961).
(24) C. M. Venkatachalam and G. N. Ramachandran, *Conform. Biopolym., Pap. Int. Symp.*, 83 (1967).

Table VII. Bond Distances (Å)

	(a) Boc-Gly-Aib-OH		
C1-N2	1.332 (1)	C1-O1	1.235 (1)
C1-C1A	1.523 (2)	C1A-N1	1.441 (2)
C2-C2A	1.538 (2)	C2-O2'	1.209 (1)
C2-O2''	1.314 (2)	C2A-C2B1	1.535 (2)
C2A-C2B2	1.524 (2)	C2A-N2	1.466 (2)
C3-N1	1.360 (2)	C3-O3	1.208 (2)
C3-O4	1.346 (1)	C4-O4	1.468 (1)
C4-C5	1.516 (2)	C4-C6	1.517 (2)
C4-C7	1.518 (2)		
	(b) Boc-Leu-Aib-Pro-OH		
C1-C1A	1.576 (12)	C1-N2	1.407 (12)
C1-O1	1.191 (11)	C1A-C1B	1.551 (12)
C1A-N1	1.419 (11)	C1B-C1G	1.580 (14)
C1D1-C1G	1.527 (15)	C1D2-C1G	1.500 (16)
C2-C2A	1.527 (13)	C2-N3	1.293 (10)
C2-O2	1.261 (10)	C2A-C2B1	1.551 (14)
C2A-C2B2	1.504 (13)	C2A-N2	1.500 (12)
C3-C3A	1.541 (15)	C3-O3'	1.209 (12)
C3-O3''	1.303 (12)	C3A-C3B	1.505 (13)
C3A-N3	1.483 (12)	C3B-C3G	1.491 (15)
C3D-C3G	1.561 (14)	C3D-N3	1.480 (12)
C4-N1	1.338 (11)	C4-O4	1.201 (13)
C4-O5	1.355 (12)	C5-C7	1.476 (15)
C5-C6	1.563 (17)	C5-C8	1.476 (14)
C5-O5	1.466 (10)		
	(c) Boc-Leu-Aib-Pro-OBzl		
C1-C1A	1.533 (3)	C1-N2	1.341 (3)
C1-O1	1.234 (3)	C1A-C1B	1.527 (3)
C1A-N1	1.451 (3)	C1B-C1G	1.523 (4)
C1D1-C1G	1.532 (5)	C1D2-C1G	1.507 (5)
C2-C2A	1.546 (3)	C2-N3	1.346 (4)
C2-O2	1.231 (3)	C2A-C2B1	1.538 (4)
C2A-C2B2	1.531 (4)	C2A-N2	1.457 (3)
C3-C3A	1.505 (5)	C3-O3''	1.336 (4)
C3-O3'	1.187 (4)	C3A-C3B	1.537 (5)
C3A-N3	1.463 (3)	C3B-C3G	1.497 (6)
C3D-C3G	1.524 (5)	C3D-N3	1.463 (4)
C4-N1	1.350 (3)	C4-O4	1.213 (3)
C4-O5	1.343 (3)	C5-C6	1.510 (5)
C5-C7	1.523 (4)	C5-C8	1.516 (4)
C5-O5	1.482 (3)	C9-C10	1.502 (5)
C9-O3''	1.459 (5)	C10-C11	1.387 (5)
C10-C15	1.383 (4)	C11-C12	1.356 (6)
C12-C13	1.380 (6)	C13-C14	1.372 (6)
C14-C15	1.373 (5)		
	(d) Boc-Pro-Aib-Ala-Aib-OBzl		
C1-C1A	1.472 (8)	C1-N2	1.345 (7)
C1-O1	1.231 (5)	C1A-C1B	1.571 (9)
C1A-N1	1.457 (6)	C1B-C1C	1.520 (9)
C1C-C1D	1.505 (9)	C1D-N1	1.471 (6)
C2-C2A	1.574 (8)	C2-N3	1.370 (8)
C2-O2	1.206 (7)	C2A-C2B1	1.574 (8)
C2A-C2B2	1.491 (8)	C2A-N2	1.477 (8)
C3-C3A	1.527 (8)	C3-N4	1.321 (8)
C3-O3	1.223 (7)	C3A-C3B	1.538 (9)
C3A-N3	1.454 (7)	C4-C4A	1.525 (8)
C4-O4''	1.311 (5)	C4-O4'	1.210 (5)
C4A-C4B2	1.491 (8)	C4A-C4B1	1.530 (10)
C4A-N4	1.453 (7)	C5-N1	1.338 (7)
C5-O5	1.207 (6)	C5-O6	1.369 (6)
C6-C7	1.501 (9)	C6-C8	1.539 (9)
C6-C9	1.505 (10)	C6-O6	1.478 (7)
C10-C11	1.399 (8)	C10-O4''	1.474 (7)
C11-C12	1.395 (10)	C11-C16	1.357 (10)
C12-C13	1.463 (10)	C13-C14	1.232 (13)
C14-C15	1.221 (13)	C15-C16	1.352 (11)

weaker second hydrogen bond results from the amino group of Aib[4] donating a proton to the proline carbonyl oxygen (N–O distance 3.10 Å). The helical conformation does not continue through the fourth residue (Aib), probably due to the limited intramolecular constraints; its torsion angles, however, are equal in magnitude but opposite in sign from that of Aib[2]. These observed values of ϕ and ψ agree remarkably well with the predicted values for an Aib residue[7] and are midway between the expected values for an α-helix and a 3_{10}-helix. The pitch of the helix and the number of residues per turn, calculated on the basis of the ϕ and ψ torsion angles, are nearly constants for each residue and are midway between the expected value for an α-helix and a 3_{10}-helix.

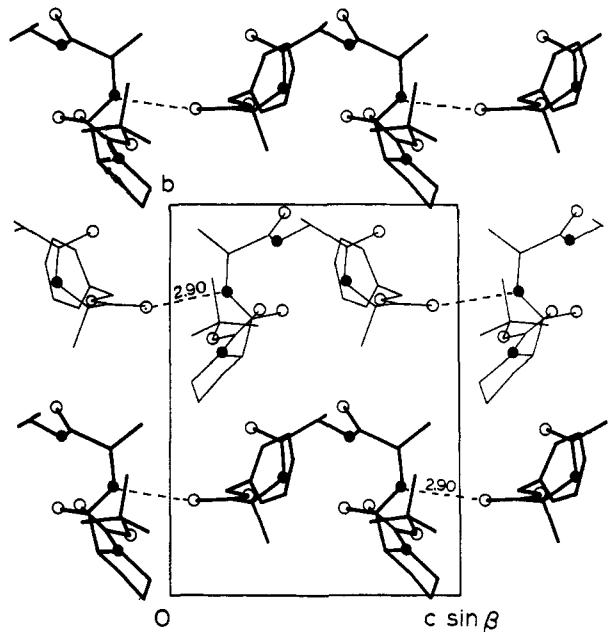

Figure 5. Packing of molecules of Boc-Pro-Aib-Ala-Aib-OBzl in the unit cell.

The conformation of the backbone of Boc-Leu-Aib-Pro-OH can best be described as a chain reversal with the Leu and Aib residues at the corners of the bend. Since proline is the (i + 4)th residue, an intramolecular hydrogen bond across the bend is not possible. Interestingly enough, the conformation of Boc-Leu-Aib-Pro-OBzl is quite different from that of the acid form and can best be described as semiextended with the Pro and Aib residues producing a kink in the backbone. A comparison of the torsion angles (Table IX) of the two molecules reveals that the conformations of the Aib and Pro residues are nearly the same. The difference in overall conformation results primarily from the rotation of 154.6° of the ψ angle while a smaller perturbation results from the rotation of 21.8° of the ϕ angle of the Leu residue. Since there are no intramolecular forces such as hydrogen bonding to stabilize the conformation, there is no reason to suspect that one conformation is of lower energy than that of the free acid. It is likely that the differences in conformation result from an energetically more favorable packing of the benzyl ester in the unit cell compared to that of the free acid. Therefore, this situation can be viewed as a case in which the energetics of the packing select from conformations of nearly equal energy.

With the exception of the two hydrogen bonds of the tetrapeptide which have already been discussed, no intramolecular hydrogen bonding exists in the other three structures. However, the intermolecular hydrogen-bonding schemes are quite different in the four crystals.

Only one intermolecular hydrogen bond exists between tetrapeptide molecules. This occurs between the amino group of Aib[2] and the carbonyl oxygen of Aib[4] (N–O distance 2.92 Å) of a translationally related molecule and produces infinite chains of hydrogen-bonded peptide molecules parallel to the *c* axis. The packing of these molecules is illustrated in Figure 5.

The amino groups of Leu and Aib of Boc-Leu-Aib-Pro-OH donate protons to O_3' (2.98 Å) and O_2 (2.86 Å), respectively, of a symmetry related molecule. Pairs of molecules are also hydrogen bonded together by means of the water molecule, which forms a link between O_3'' of one molecule (2.68 Å) and O_1 of a neighboring molecule (2.72 Å). The packing of molecules of Boc-Leu-Aib-Pro-OH and the intermolecular hydrogen-bonding

Table VIII. Bond Angles (Deg)

	(a) Boc-Gly-Aib-OH		
O1-C1-N2	121.4 (1)	C1A-C1-N2	117.1 (1)
C1A-C1-O1	121.5 (1)	N1-C1A-C1	111.3 (1)
O2'-C2-C2A	123.6 (1)	O2''-C2-C2A	112.5 (1)
O2''-C2-O2'	123.8 (1)	C2B1-C2A-C2	106.8 (1)
C2B2-C2A-C2	110.5 (1)	N2-C2A-C2	109.1 (1)
C2B2-C2A-C2B1	111.5 (1)	N2-C2A-C2B1	107.5 (1)
N2-C2A-C2B2	111.3 (1)	O3-C3-N1	125.1 (1)
O4-C3-N1	109.1 (1)	O4-C3-O3	125.8 (1)
C5-C4-O4	109.2 (1)	C6-C4-O4	102.5 (1)
C7-C4-O4	110.6 (1)	C6-C4-C5	111.3 (1)
C7-C4-C5	112.3 (1)	C7-C4-C6	110.5 (1)
C3-N1-C1A	118.3 (1)	C1-N2-C2A	121.6 (1)
C4-O4-C3	120.3 (1)		
	(b) Boc-Leu-Aib-Pro-OH		
N2-C1-C1A	110.6 (6)	O1-C1-C1A	125.5 (7)
O1-C1-N2	123.3 (6)	C1B-C1A-C1	109.8 (7)
N1-C1A-C1	106.8 (6)	N1-C1A-C1B	111.7 (6)
C1A-C1B-C1G	111.8 (7)	C1D1-C1G-C1D2	112.8 (8)
C1B-C1G-C1D2	111.3 (8)	C1B-C1G-C1D1	106.9 (8)
N3-C2-C2A	123.1 (7)	O2-C2-C2A	115.5 (6)
O2-C2-N3	121.4 (6)	C2B1-C2A-C2	106.0 (7)
C2B2-C2A-C2	112.8 (7)	N2-C2A-C2	112.0 (7)
C2B2-C2A-C2B1	111.3 (7)	N2-C3A-C2B1	105.7 (7)
N2-C2A-C2B2	108.7 (7)	O3'-C3-C3A	121.7 (7)
O3''-C3-C3A	112.2 (7)	O3''-C3-O3'	125.7 (7)
C3B-C3A-C3	111.9 (8)	N3-C3A-C3	109.8 (7)
N3-C3A-C3B	105.0 (7)	C3A-C3B-C3G	100.7 (8)
N3-C3D-C3G	99.8 (7)	C3B-C3G-C3D	107.1 (8)
O4-C4-N1	123.3 (7)	O5-C4-N1	108.8 (7)
O5-C4-O4	127.8 (7)	C6-C5-C7	108.4 (8)
C8-C5-C7	112.6 (8)	O5-C5-C7	103.8 (7)
C8-C5-C6	107.6 (8)	O5-C5-C6	110.2 (7)
O5-C5-C8	114.1 (7)	C4-N1-C1A	120.7 (6)
C2A-N2-C1	119.0 (6)	C3D-N3-C2	129.4 (6)
C3A-N3-C2	118.7 (6)	C3A-N3-C3D	111.7 (6)
C5-O5-C4	118.9 (6)		
	(c) Boc-Leu-Aib-Pro-OBzl		
N2-C1-C1A	116.9 (2)	O1-C1-C1A	120.5 (1)
O1-C1-N2	122.5 (1)	C1B-C1A-C1	111.3 (2)
N1-C1A-C1	111.9 (2)	N1-C1A-C1B	109.7 (2)
C1G-C1B-C1A	115.0 (2)	C1D1-C1G-C1B	110.4 (2)
C1D2-C1G-C1B	112.7 (2)	C1D2-C1G-C1D1	111.0 (2)
N3-C2-C2A	120.4 (2)	O2-C2-C2A	119.9 (2)
O2-C2-N3	119.2 (2)	C2B1-C2A-C2	106.2 (2)
C2B2-C2A-C2	109.6 (2)	N2-C2A-C2	113.2 (2)
C2B2-C2A-C2B1	110.3 (2)	N2-C2A-C2B1	108.3 (2)
N2-C2A-C2B2	109.2 (2)	O3''-C3-C3A	109.2 (2)
O3'-C3-C3A	126.6 (2)	O3'-C3-O3''	124.1 (2)
C3B-C3A-C3	111.1 (2)	N3-C3A-C3	110.8 (2)
N3-C3A-C3B	103.5 (2)	C3G-C3B-C3A	103.5 (3)
N3-C3G-C3G	104.2 (2)	C3D-C3G-C3B	103.3 (3)
O4-C4-N1	123.1 (2)	O5-C4-N1	110.7 (2)
O5-C4-O4	126.2 (2)	C7-C5-C6	111.4 (2)
C8-C5-C6	113.2 (2)	O5-C5-C6	109.4 (2)
C8-C5-C7	110.7 (2)	O5-C5-C7	101.9 (2)
O5-C5-C8	109.6 (2)	O3''-C9-C10	108.8 (3)
C11-C10-C9	122.9 (3)	C15-C10-C9	119.4 (3)
C15-C10-C11	117.7 (3)	C12-C11-C10	121.3 (3)
C13-C12-C11	120.6 (3)	C14-C13-C12	119.0 (3)
C15-C14-C13	120.4 (3)	C14-C15-C10	120.9 (3)
C4-N1-C1A	119.1 (2)	C2A-N2-C1	123.1 (2)
C3A-N3-C2	118.4 (2)	C3O-N3-C2	130.6 (2)
C3D-N3-C3A	111.0 (2)	C5-O5-C4	120.6 (2)
C9-O3''-C3	119.3 (2)		
	(d) Boc-Pro-Aib-Ala-Aib-OBzl		
N2-C1-C1A	115.9 (4)	O1-C1-C1A	122.3 (4)
O1-C1-N2	121.7 (4)	C1B-C1A-C1	113.2 (4)
N1-C1A-C1	116.1 (4)	N1-C1A-C1B	102.2 (4)
C1C-C1B-C1A	101.9 (4)	C1B-C1C-C1D	106.9 (5)
N1-C1D-C1C	102.4 (4)	N3-C2-C2A	113.1 (4)
O2-C2-C2A	121.4 (4)	O2-C2-N3	124.7 (4)
C2B1-C2A-C2	108.4 (4)	C2B2-C2A-C2	108.2 (4)
N2-C2A-C2	110.6 (4)	C2B2-C2A-C2B1	111.1 (4)
N2-C2A-C2B1	109.3 (4)	N2-C2A-C2B2	109.2 (4)
N4-C3-C3A	116.8 (4)	O3-C3-C3A	120.6 (4)
O3-C3-N4	122.6 (4)	C3B-C3A-C3	109.4 (4)
N3-C3A-C3	115.4 (4)	N3-C3A-C3B	106.9 (4)
O4''-C4-C4A	113.4 (4)	O4'-C4-C4A	123.0 (4)
O4'-C4-O4''	123.1 (4)	C4B2-C4A-C4	109.9 (5)
C4B1-C4A-C4	105.2 (4)	N4-C4A-C4	110.5 (4)
C4B1-C4A-C4B2	110.5 (5)	N4-C4A-C4B2	113.1 (4)
N4-C4A-C4B1	107.3 (4)	O5-C5-N1	124.9 (4)
O6-C5-N1	110.7 (3)	O6-C5-O5	124.4 (4)
C8-C6-C7	111.7 (5)	C9-C6-C7	113.6 (5)
O6-C6-C7	111.8 (4)	C9-C6-C8	108.3 (5)
O6-C6-C8	101.4 (4)	O6-C6-C9	109.2 (4)
O4''-C10-C11	109.9 (5)	C12-C11-C10	122.4 (5)
C16-C11-C10	122.3 (5)	C16-C11-C12	115.3 (5)
C13-C12-C11	119.1 (6)	C14-C13-C12	113.3 (6)
C15-C14-C13	132.9 (7)	C16-C15-C14	116.9 (7)
C15-C16-C11	122.4 (6)	C1A-N1-C10	114.2 (3)
C5-N1-C10	125.7 (3)	C5-N1-C1A	118.9 (3)
C2A-N2-C1	123.1 (4)	C3A-N3-C2	119.3 (4)
C4A-N4-C3	122.1 (4)	C6-O6-C5	119.9 (3)
C10-O4''-C4	115.3 (4)		

Table IX. Torsion Angles (Deg) for the Four Peptides[a]

residue	ϕ	ψ	ω	χ^1	χ^2	χ^3	χ^4
Gly	89.5 (1)	165.1 (1)	−175.7 (1)				
Aib-OH	46.8 (1)	44.8 (1)					
Leu	−84.4 (10)	163.1 (7)	−177.9 (7)	−67.1 (9)	−179.2 (8)		
Aib	53.3 (10)	37.3 (12)	176.9 (8)				
Pro-OH	−71.3 (10)	160.8 (8)		34.1 (10)	−40.2 (10)	29.8 (10)	−7.4 (9)
Leu	−62.6 (3)	−42.3 (3)	169.6 (2)	−72.2 (2)	165.3 (2)		
Aib	52.8 (3)	46.1 (3)	169.2 (2)				
Pro-OBzl	−74.4 (3)	161.7 (2)	176.3 (3)	31.0 (3)	−38.2 (4)	31.0 (4)	−11.7 (3)
Pro	−53.3 (6)	−38.0 (6)	−176.5 (5)	−30.0 (5)	35.7 (6)	−26.6 (6)	6.5 (6)
Aib	−50.4 (6)	−40.6 (6)	−173.6 (5)				
Ala	−91.4 (6)	−11.1 (7)	−175.9 (5)				
Aib-OBzl	44.3 (7)	42.4 (6)	180.0 (5)				

	θ^2	θ^1	ω_0	θ^3	θ^4
Boc-Gly-Aib-OH	73.6 (1)	170.5 (1)	−159.4 (1)		
Boc-Leu-Aib-Pro-OH	61.6 (10)	178.5 (7)	171.5 (7)		
Boc-Leu-Aib-Pro-OBzl	61.5 (3)	−172.4 (2)	−174.3 (2)	−118.4 (3)	54.4 (5)
Boc-Pro-Aib-Ala-Aib-OBzl	68.6 (6)	−178.2 (4)	−174.3 (4)	165.0 (5)	−74.6 (9)

[a] The torsion angles: ω_0 measures the rotation about the urethane bond which links the peptide to Boc; θ^1 is a measure of the torsion angle C_t-O-CO-N_1 while θ^2 corresponds to the torsion angle H_3C-C_t-O-CO; the two torsion angles θ^3 and θ^4 correspond to the angles CO-O-CH_2-C_{benzyl} and O-CH_2-C_{benzyl}-CH_{benzyl} of the benzyl group.

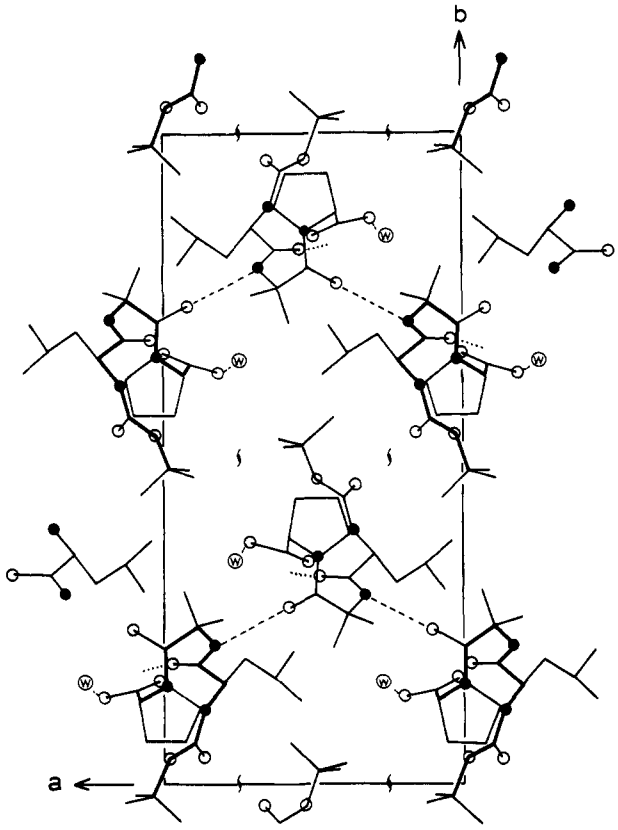

Figure 6. Packing of molecules of Boc-Leu-Aib-Pro-OH in the unit cell.

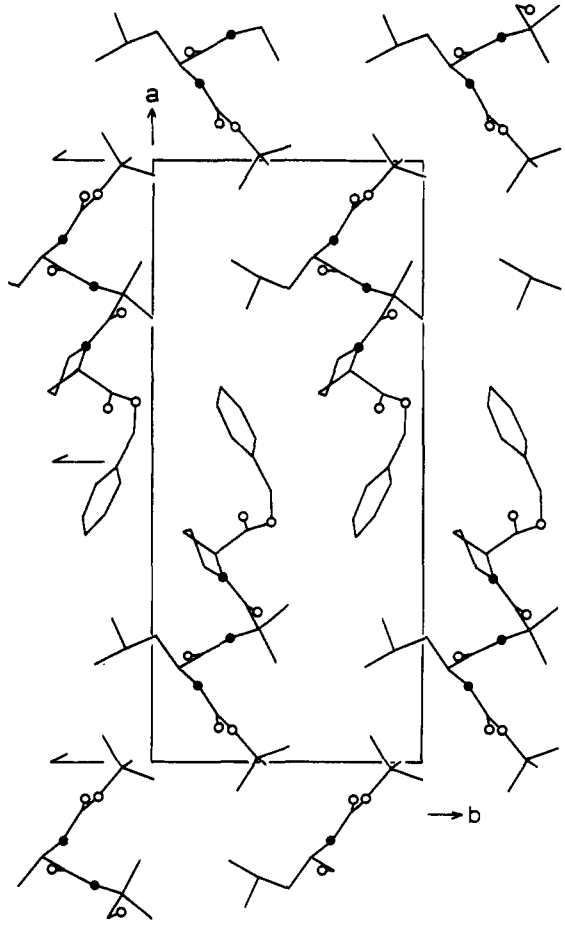

Figure 7. Packing of molecules of Boc-Leu-Aib-Pro-OBzl in the unit cell viewed parallel to the direction of the hydrogen bonds which link adjacent unit cells together.

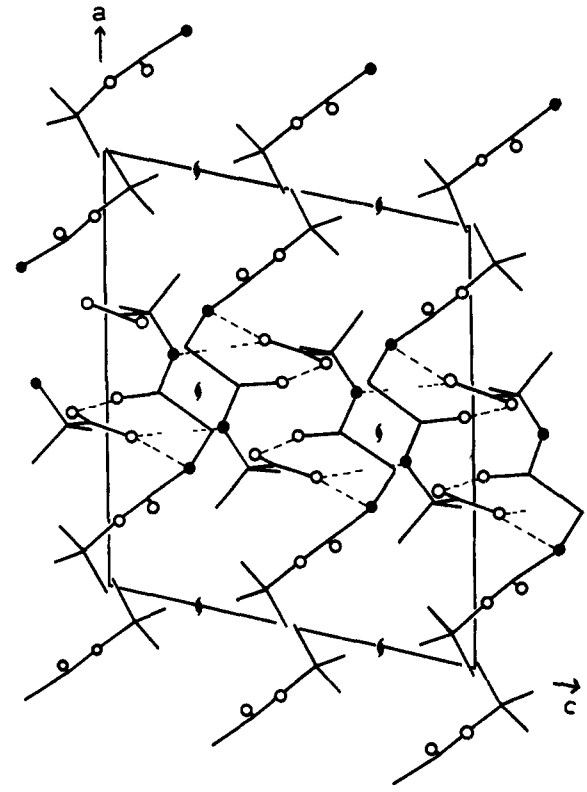

Figure 8. Packing of molecules of Boc-Gly-Aib-OH in the unit cell.

scheme are illustrated in Figure 6, where the contents of the unit cell are shown projected upon the *ab* plane.

The packing and intermolecular hydrogen-bonding scheme of Boc-Leu-Aib-Pro-OBzl, illustrated in Figure 7, are considerably simpler than that of the previous structure. In this case, hydrogen bonds exist between the amino groups of Leu and Aib and the carbonyl oxygen atoms of the same residues but in a molecule of an adjacent unit cell with distances of 3.03 and 3.12 Å, respectively. The effect of this hydrogen-bonding scheme is to produce infinite chains of hydrogen-bonded peptide molecules parallel to the *c* axis. Hydrophobic channels also exist in the crystal and consist of the methyl groups of Boc and of the Aib residue, the Leu and Pro side chains, and finally the benzyl group.

The intermolecular hydrogen bonding of Boc-Gly-Aib-OH is illustrated in Figure 8. The amino groups of both Gly and Aib donate a proton to O_2' with distances of 3.08 and 2.98 Å, respectively. An additional hydrogen bond also exists between the acidic proton of the carbonyl group and O_1 (2.62 Å).

The conformation of the side chains of the Leu residues is fully extended and is the most frequently observed one.[25] The results of this study provide three observations of the conformation of the pyrrolidine ring of proline. Asymmetry parameters[26] for the acidic form and the benzyl ester of the tripeptide, respectively, were calculated to be 10.2 and 14.5 for a mirror plane passing through the β-carbon atom and 7.2 and 0.1 for a twofold axis passing through the nitrogen atom. Values of 7.9 and 8.9 were obtained for the tetrapeptide. These values show that the pyrrolidine ring can be described as being nearly midway between the half chair and envelope forms for the acidic form of the tripeptide as well as for the tetrapeptide. However, these parameters also reveal that the conformation of this ring in the benzyl ester of the tripeptide is that of a half chair. Careful examination of the torsion angles reveals additionally that the γ-carbon atom is exo in the tetrapeptide but endo in both tripeptides.

The conformation of the Boc group in all four cases is fully extended; that is, a methyl group is trans to the sp^2 carbon atom

(25) E. Benedetti, *Pept., Proc. Am. Pept. Symp., 5th*, 257-273 (1977).
(26) W. L. Duax, C. M. Weeks, and D. C. Rohrer, *Top. Stereochem.*, **9**, 281-383 (1976).

of the carbonyl group (measured by the torsion angle θ^2) and the nitrogen is trans to the *tert*-butyl group (θ^1). In addition, the urethane bond (ω_0) in the tetrapeptide, linking Boc and Pro, is trans, in contrast to the cis junction of all but one of the published structures containing the sequence Boc-L-Pro-X.[25,27] However, the cis linkage is inconsistent with the observed hydrogen-bonding scheme, suggesting that the conformation of Boc is selected by the intramolecular hydrogen bonds.

Discussion

This study of four linear fragment peptides of alamethicin (Ac-Aib-Pro-Aib-Ala-Aib-Ala-Gln-Aib-Val-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-Glu-Gln-Phol)[5] provides five independent observations of the conformation of an Aib residue. The results are in excellent agreement with theoretical studies (compare Tables IV and IX) and clearly demonstrate that the crystallographically determined average value of ±(50, 42) for (ϕ, ψ) for this residue is a very low-energy form.

The fact that the magnitudes of the five sets of value have a range of only 9 degrees is even more remarkable when one considers the following facts: (1) all the peptides are linear and therefore have no additional constraints restricting their conformation; (2) the Aib residue is covalently bound to different groups in three of the four cases; (3) one of the peptides crystallizes as a monohydrate while the other three are anhydrous; (4) the tetrapeptide contains two intramolecular hydrogen bonds while the remainder contain none; (5) packing of the tetrapeptide molecules in the crystal lattice produces a single intermolecular hydrogen bond between molecules while a more complicated intermolecular hydrogen-bonding scheme exists for the other three. These points strongly suggest that the observed magnitudes of ϕ and ψ for Aib are highly preferred. Furthermore, the totally different crystalline environments indicate that crystal-packing forces have not played an important role in determining conformations for this residue.

Descriptions of the structures of four other peptides containing Aib have appeared in the literature. In one case, however, the structure reported was that of a cyclic tetrapeptide dihydrochlamydocin;[28] not surprisingly, the observed conformation (ϕ, ψ = 71.8, −63.7) of Aib is significantly different. The 15–24° variations from 180° of the ω torsion angles is indicative of the amount of strain in this cyclic tetrapeptide. The second example is a linear tetrapeptide ((*Z*)-Aib-Pro-Aib-Ala-OMe) comprising residues 1–4 of alamethicin and containing two residues of Aib.[9] Although the first residue adopts the expected conformation, Aib[3] is somewhat removed from the minimum energy position and in fact is close to the (ϕ, ψ) value of Ala[3] observed in the present study. This suggests that the third residue in the proline containing "helix" must deviate from the ideal (ϕ, ψ) value of (−50, −42) in order to maintain the intramolecular hydrogen-bonding scheme. Two values are observed for the Aib corresponding to residue-3 of alamethicin. In the study of Shamala et al.,[9] a value of (−72, −11) was found, while (−50, −41) is observed in this study. This suggests that the local environment -Pro-Aib-Ala, which is identical in the two cases, does not restrict the Aib residue to a single conformational choice. The torsional rotations assumed must, therefore, be influenced by longer range intramolecular interactions and intermolecular forces such as hydrogen bonding.

The structure of both the pentamer of Aib[10] which assumes a 3_{10}-helix and (*Z*)-Aib-Pro-NHMe[11] offers additional observations as molecules with both right- and left-handed conformations are observed. The total of 17 experimental observations are compared with the calculated values previously published[7] in Figure 9. With the exception of the Aib residue in dihydrochlamydocin, all of the values cluster near the two symmetrical minima (ϕ, ψ = −55, −40 or 55, 40) previously calculated. Only one (ϕ = −72, ψ = −11) of these latter 16 values lies outside the 0.5 kcal above the minimum contour[29] and within the 1.0-kcal contour line. This

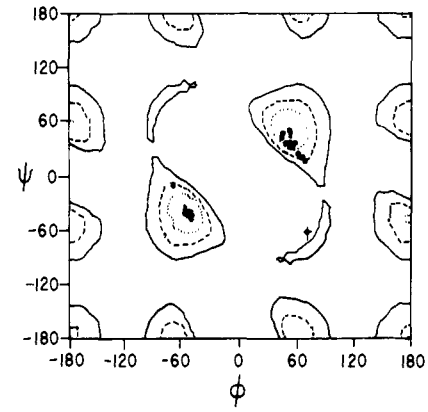

Figure 9. Potential energy plot of Ac-Aib-NH-CH_3 compared with crystal structure observations for Aib (o). Contours drawn at 0.5, 1.0, and 2.0 kcal/mol above the potential minima. Values outside contours are at ϕ = 72, ψ = −64 from cyclic tetrapeptide dihydrochlamydocin.[28]

Table X. Calculated Conformations of Ac-Aib-Ala-Aib-NH-CH_3[a]

conformer	ϕ_1	ψ_1	ϕ_2	ψ_2	ϕ_3	ψ_3	kcal/mol above minima
R,R	51	−37	−67	−43	−56	−32	0
L,L	47	34	49	54	50	43	0.2
R,L-A	−57	−49	−79	−30	46	41	0.4
R,L-B	−57	−49	−109	58	46	41	0.4
crystal structure -Aib-Ala-Aib-	−50	−41	−91	−11	44	42	

[a] These are the minimum energy conformers calculated from the systematic exploration of conformations near the energy minima for Aib (see Table VI). [b] R,R signifies that the conformer was selected by exploration with the two Aib residues near the right-handed helical minimum.

agreement lends strong experimental support to the validity of the Kitaygorodsky parameterization used. Pletnev et al.[8a] calculated a favorable C_7^{ax} conformation (ϕ = −64, ψ = 71, and the symmetrical conformer) especially in nonpolar media, which Burgess and Leach[8b] also found by PCILO calculations, but which they concluded was at least 5 kcal/mol less stable than the helical conformers from empirical calculations. Aubry et al.[30] have interpreted infrared spectra of acetylaminoisobutyric acid methylamide in CCl_4 to indicate a predominance of C_5 (ϕ = ψ = 180) and C_7 (ϕ, ψ = −70, 70 or 70, −70) conformers even though the crystal structure of this amino acid derivative has ϕ = −55.5° and ψ = −39.3°. The C_7 conformer would be similar to the conformation of the Aib seen in dihydrochlamydocin but is not within the allowed region[31] according to the Kitaygorodsky potential (greater than 2 kcal/mol above the potential minima). A relatively small increase (15°) in ϕ or ψ from the C_7 values would allow them to enter a region considered allowed, and this may be the conformer which is attributed to C_7 by Aubry et al.,[30] although it is also likely that other conformers may be capable of hydrogen bonding and contribute the appropriate IR bands which they have observed. Rao et al.[31] have observed similar IR bands for (*Z*)-Aib-Aib-OMe and (*Z*)-Aib-Ala-OMe in CCl_4. The fact that none of the 16 Aib residues observed in linear peptides assume a value near the C_7 conformation probably indicates that this conformer is not favored, and minor differences in param-

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(28) J. L. Flippen and I. L. Karle, *Biopolymers*, **5**, 1081–1092 (1976).
(29) Calculated kcal/mol must be calibrated by comparison with experimental values. On the basis of the protein data base, conformations calculated with the Kitaygorodsky potential to be greater than 2 kcal/mol above the minimum are unlikely (see G. R. Marshall, H. E. Bosshard, W. Vine, J. D. Glickson, and P. Needleman, *Recent Adv. Renal Physiol. Pharmacol.* [*Proc. Ann. A. N. Richards Symp.*], *15th*, 215–226 (1974), for further discussion as well as Reference 7).
(30) A. Aubry, J. Protas, G. Boussard, M. Marraud, and J. Neel, *Biopolymers*, **17**, 1693 (1978).
(31) C. P. Rao, R. Nagaraj, C. N. R. Rao, and P. Balaram, *Biochemistry*, **19**, 425 (1980).

eterization of the potential functions used by the different groups may explain the difference in their predictions, especially since these compounds are sterically hindered and sensitive to choice of van der Waals parameters. The structures of the two tripeptides Boc-Leu-Aib-Pro-OX (X = H, Bzl) and the published tetrapeptide,[9] (Z)-Aib-Pro-Aib-Ala-OMe, provide three examples of Aib residues preceding a proline. Theoretical calculations (Moore and Marshall, in preparation) suggest only a slight preference for the positive values of ϕ and ψ for Aib residues preceding a proline and both combinations are observed experimentally. Systematic calculation of the possible conformers of acetyl-Aib-Ala-Aib methylamide at 11.25° increments gave four conformers within 0.5 kcal/mol of the minimum as shown in Table X. Conformer R,L-A is very similar to the crystal structure observed for the fragment-Aib-Ala-Aib of the tetrapeptide, averaging only 8° deviation from the observed structure. This further supports the conclusion that the primary determinants of conformation in these molecules are intramolecular with crystal-packing forces selecting between energetically similar conformers. It should, therefore, be feasible to apply the constraints introduced by Aib residues to limit the possible conformations available to the alamethicin molecule. In addition, other antibiotics such as antiamoebin,[32] emerimicin,[33] and suzukacillin[34] have been shown to contain Aib residues. The name of peptaibophol antibiotics has been proposed[32] for this class which contains phenylalaninol as well as several residues of Aib. The unique properties associated with α-methyl substitution have been recognized by natural selection and have resulted in this class of compounds with unique membrane properties.

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Supplementary Material Available: Listings of atomic coordinates, bond distances and angles for the nonhydrogen and hydrogen atoms, and a tabulation of observed and calculated structure factors (71 pages). Ordering information is given on any current masthead page.

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Synthesis and Reactions of Simple 3(2*H*)-Furanones

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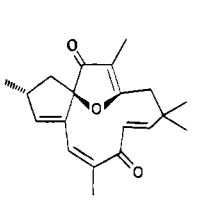
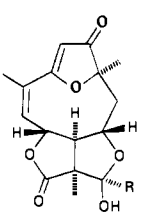
Abstract: Interest in the total synthesis of natural product antitumor agents which have as a central structural element the 3(2*H*)-furanone ring system has led to the development of an efficient general synthesis of a variety of simple 3(2*H*)-furanones. The strategy involves aldol condensation of aldehydes with the enolate derived from 3-methyl-3-(trimethylsiloxy)-2-butanone (**35**) followed by Collins oxidation to afford the 1,3-diketone. Acid-catalyzed cyclization–dehydration then leads to the corresponding 3(2*H*)-furanones. The availability of this facile approach to the 3(2*H*)-furanone ring system provided the opportunity to explore the chemistry of this increasingly important heterocycle. Three reactions were selected for initial study; they were (a) alkylation, (b) conjugate addition of organocuprate reagents, and (c) reaction with sulfur nucleophiles. The results of the latter vis-ā-vis the mode of action of 3(2*H*)-furanone antitumor agents is discussed.

Introduction and Background

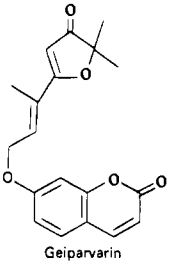
During the course of studies directed at devising a viable synthetic approach to jatrophone[2] (**1**) and related antitumor agents such as the eremantholides[3] (A, B, and C) (**2**) and geiparvarin[4] (**3**), each of which possesses as a central structural element the 3(2*H*)-furanone ring, we have had occasion to explore the synthesis and chemistry of a number of simple 3(2*H*)-furanones. We report here the results of that study.



Jatrophone
1



Eremantholide
2a: R = *i*-Pr
2b: R = *sec*-Bu
2c: R = CMe=CH$_2$



Geiparvarin
3

Our interest in simple 3(2*H*)-furanones was threefold. First, selection of the above synthetic targets demanded the availability of an efficient and hopefully general strategy for construction of

(1) Camille and Henry Dreyfus Teacher-Scholar, 1978–1983, and National Institutes of Health (National Cancer Institute) Career Development Awardee, 1980–1985.

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