Proton Magnetic Resonance Spectra of Amino-acids and Peptides relevant to Wool Structure. Part IV.[1] Relative Residence Times of Dipeptides and Tripeptides of Phenylalanine and Tyrosine

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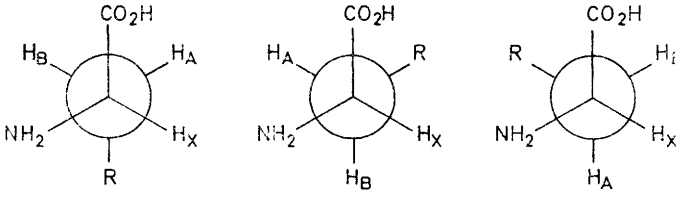
100 MHz High resolution ^{1}H n.m.r. spectra have been recorded from acidic (pD 0.6—1.4) and basic (pD 12.3—13.1) D_2O solutions of the dipeptides Gly-Phe, Ala-Phe, Val-Phe, Met-Phe, Leu-Phe, Phe-Leu, Phe-Val, Phe-Tyr, His-Phe, Gly-Tyr, Ala-Tyr, Val-Tyr, L-Leu-Tyr, D-Leu-Tyr, Trp-Tyr, Trp-Gly, and Tyr-Val, within the range 300—360 K. Iterative analyses of ABC/ABX systems have yielded coupling constants from which relative populations of C_α–C_β rotamers have been derived. 100 and 220 MHz ^{1}H spectra have similarly been analysed for the basic and acidic D_2O solutions of the tripeptides Gly-Phe-Met, Met-Phe-Gly, Gly-Phe-Ala, Gly-Phe-Phe, Gly-Tyr-Gly, and Val-Tyr-Val, within the range 280—360 K. In acid, the *trans*-rotamer (ring opposed to carboxyl or *C*-terminal peptide group) is favoured for dipeptides of the types X-L-Phe and X-L-Tyr and for the tripeptides Gly-Phe-Ala and Gly-Tyr-Gly; in base, the *trans*-rotamer predominates in most peptides.

DETAILED analysis of the ^{1}H n.m.r. spectra of small peptides can not only be informative about side-chain and backbone rotational isomerism but is also a prerequisite for interpretation of the more complex protein spectra exhibited by wool.[2-5] For the side-chain C_α–C_β bond, the α-methine and β-methylene hydrogens often constitute an ABC/ABX spin system; extraction of the vicinal coupling constants J_{BC} and J_{AC} enables the rotamer populations *a*—*c* of the classical staggered rotamers (I)—(III) to be evaluated by the Pachler procedure.[6] In basic solution, rotamer populations of amino-acids and dipeptides may equalize, diverge, or be invariant to increases in temperature.[1]

For analysis of conformation about the N–C_α backbone, Bystrov [7] has proposed a Karplus-type angular dependence for the vicinal N*H*–C_α*H* coupling constant. Several values of the dihedral angle are consistent with



(I) (II) (III)

the measured coupling constant, but the ambiguity may be reduced by utilizing plausible values of ϕ and ψ derived from potential energy calculations.[8]

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EXPERIMENTAL

Materials.—Samples of the dipeptides glycyl-L-phenylalanyl-L-alanine, glycyl-L-phenylalanyl-L-phenylalanine, glycyl-L-tyrosyl-glycine, and L-valyl-L-tyrosyl-L-valine were purchased from Sigma Chemical Co. Ltd. Mann Research Laboratories supplied L-methionyl-L-phenylalanyl-L-methionine and L-methionyl-L-phenylalanyl-glycine. Solvents were deuterium oxide (99.7% isotopic purity) from Prochem Ltd., acidified distilled water, deuterium chloride (20% solution in D_2O) from Koch–Light Ltd., and sodium deuterioxide (30% solution in D_2O) from CIBA (ARL) Ltd.

The internal standard was t-butyl alcohol (B.D.H.). The acidity, pD, adjusted by dropwise addition of either DCl or NaOD, was estimated [9] by adding 0.4 to the pH measured on an EIL meter, model 23A, with a Jena dual (glass–KCl reference) electrode, model 9259/81.

Spectra.—^{1}H N.m.r. spectra of 4—5% w/w peptide solutions were recorded on a JEOL MH-100 or a Varian HR-220 spectrometer in the internal-lock mode. Although the methyl signal of 2% w/w t-butyl alcohol moves upfield with increasing temperature, it was preferred to sodium 4,4-dimethyl-4-silapentane-1-sulphonate as the internal reference because of the absence of absorptions in the methylene and methine proton regions. On the MH-100 instrument, the probe temperature was measured from the separation of ethanediol resonances. Typical spectrometer conditions were: sweep width 108—1080 Hz, sweep time 250 or 500 s (low to high field scans), frequency response 5—20 Hz, and radiofrequency attenuation 10—20 db. Spectra were calibrated by generation of side-bands of the reference methyl signal from an Advance J2 audio-frequency oscillator and monitored by an Advance TC2 timer–counter.

For the HR-220 spectrometer, typical conditions were: frequency response 0.4—2.0 Hz, sweep width 100—2 500 Hz, sweep times 250 or 500 s, and radiofrequency attenuator 10—20 db. 100 Hz Sweep widths were calibrated from audio-frequency side-bands of the reference methyl signal produced by a Hewlett–Packard model 4204 signal generator and monitored by an Advance PC9A timer–counter. The probe temperature was measured to 1 K with a Comark electronic thermometer (copper–constantan thermocouple, model 1624).

Calculations.—Refinement of spectral parameters from ABX starting values by the LAME program (Mr. C. W. Haigh) was carried out on the Bradford University I.C.T. 1909 computer, as outlined previously.[1]

RESULTS AND DISCUSSION

Side-chain Rotations of Phenylalanyl-containing Di- and Tri-peptides.—The ^{1}H n.m.r. parameters and calculated rotamer populations for some phenylalanyl-containing peptides in acidic solution are listed in Table 1. Although the values of the *trans*- and *gauche*-coupling constants, J_t 13.6 and J_g 2.6 Hz, for each rotamer were originally derived [10] for amino-acids in basic solution, there is evidence that extension to acidic solution is justifiable. Now J_t and J_g depend mainly on the dihedral angle θ; J_g depends somewhat on electronegativity but J_t only slightly. Indeed, for alanine and several other compounds with ionizable groups, the average coupling constant $[\frac{1}{3}(J_t + 2J_g)]$ is almost constant over a wide pH range.[11] Also, in acid solution the serine residue of *cyclo*-L-His-L-Ser, in the conformation with all three hydrogens *gauche*,[12] has both vicinal coupling constants 2.6 Hz. Accordingly, the low pD rotamer populations in Table 1 have been calculated with the same values J_t 13.6 and J_g 2.6 Hz.[6] While small deviations from these may be expected, any changes in the vicinal coupling constants with pD and temperature are likely to reflect genuine changes in rotamer populations.

Similarity of the parameters for the five X-Phe dipeptides (X = Gly, Ala, Val, Met, and Leu) suggests that the influence of different neighbouring amino-acids is small. At 301 K, the shifts for the protons labelled B, A, and X are within 0.1 p.p.m. of the mean values of δ 1.83, 1.98, and 3.45, respectively. The similarity of the vicinal coupling constants means that the rotamer populations about the C_α–C_β bond bear a close resemblance and are little changed by an increase of temperature from 301 to 353 K. For some stereospecifically monodeuteriated *erythro*- and *threo*-phenylalanyl derivatives, Kirby and Michael [13] reported values of 9 and 5 Hz, respectively. Accordingly, for the X-Phe dipeptides, rotamer (I) (with the phenyl ring and carboxy-group *trans*) is assumed predominant, while rotamer (III) (with the phenyl ring *gauche* to the carboxy and peptide groups) is least favoured. For the Phe-X dipeptides (X = Leu, Val, and Tyr), there is no longer a downfield shift of the C_αH proton of X-Phe dipeptides caused by the *N*-terminal peptide bond and, although the charged amino-group is deshielding, the C_αH shifts are to high field of those in X-Phe dipeptides. The deceptive simplicity of the spectra at 100 MHz means that only the sum of the vicinal coupling constants can be extracted, so only rotamer populations c and $(a + b)$ may be calculated. However, the size of the vicinal coupling constants suggests that the rotamer populations about the C_α–C_β bond of phenylalanine in X-Phe and the Phe-X dipeptides are similar. For the tripeptides Gly-Phe-Ala, Met-Phe-Phe, and Met-Phe-Gly, the rotamer populations differ slightly. However, in all cases rotamer (I) predominates, in agreement with *X*-ray [14] and ^{1}H n.m.r. data [15] for the central phenylalanyl residue of Gly-Phe-Phe. Rotamer (II) is appreciably populated, while rotamer (II) (with the phenyl ring *gauche* to the peptide bonds) is least favoured. In view of the similarity of their rotamer populations, Gly-Phe-Ala and Met-Phe-Met have surprisingly different 220 MHz spectra. The Gly-Phe-Ala spectrum contain 12 resolved lines but that of Met-Phe-Met has only five; while it closely resembles an A_2X spectrum, errors would be incurred by treating it as such.[16] Increase of temper-

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TABLE 1

^{1}H N.m.r. parameters and rotamer populations of phenylalanyl-containing peptides in acidic solution

Peptide	pD	T/K	Chemical shifts [a] δ_B	δ_A	δ_X	$\delta_A - \delta_B$	Coupling constants J/Hz J_{AB}	J_{BX}	J_{AX}	Rotamer populations a	b	c
Gly–Phe	0.8	301	1.80	2.00	3.52	0.199	−14.2	8.8	5.5	0.56	0.26	0.18
		326	1.80	1.99	3.55	0.186	−14.2	8.7	5.6	0.55	0.27	0.18
		353	1.87	2.05	3.56	0.184	−14.4	8.8	5.8	0.56	0.29	0.15
Ala-Phe	0.8	301	1.80	1.97	3.41	0.170	−14.2	9.0	5.7	0.58	0.28	0.14
		326	1.83	2.00	3.52	0.165	−14.1	8.9	5.8	0.57	0.29	0.14
		353	1.91	2.07	3.55	0.163	−14.4	8.8	5.8	0.56	0.29	0.15
Val-Phe	0.7	301	1.83	1.96	3.44	0.132	−14.1	8.6	6.1	0.55	0.32	0.13
		326	1.90	2.04	3.56	0.134	−14.5	8.8	6.2	0.56	0.33	0.11
		353	1.91	2.05	3.58	0.138	−14.6	8.6	6.1	0.55	0.32	0.13
Met-Phe	0.6	301	1.85	1.98	3.43	0.126	−14.3	8.9	5.8	0.57	0.29	0.14
		327	1.92	2.05	3.54	0.123	−14.3	8.9	6.0	0.57	0.31	0.12
		353	1.95	2.08	3.58	0.124	−14.6	8.7	5.9	0.55	0.30	0.15
Leu-Phe	0.6	301	1.87	2.00	3.45	0.129	−14.2	8.9	6.0	0.57	0.31	0.12
		326	1.88	2.00	3.47	0.124	−14.3	8.9	6.0	0.57	0.31	0.12
		353	1.95	2.07	3.56	0.126	−14.6	8.9	6.1	0.57	0.32	0.11
Phe-Leu	0.7	302	1.98 [c]		3.07							
		323	2.03		3.15			14.8 [d]		0.87 [e]		0.13
		352	2.05		3.18			14.4		0.84		0.16
Phe-Val	0.9	302	1.96 [c]		3.12			14.5 [d]		0.85		0.15
		328	2.03		3.21			14.8		0.87		0.13
		338	2.04		3.23			14.8		0.87		0.13
Phe-Tyr	1.0	302	1.91		2.97			14.3		0.83		0.17
		323	1.95		3.02			14.3		0.83		0.17
Gly-Phe-Ala [b]	1.3	300	1.78	1.93	3.44	0.150	−13.9	8.4	6.4	0.53	0.35	0.12
		324	1.77	1.92	3.45	0.154	−14.0	8.1	6.4	0.50	0.35	0.15
		348	1.78	1.93	3.46	0.153	−13.9	8.2	6.3	0.51	0.34	0.15
Met-Phe-Met	0.8	293	1.85 [c]		3.42			15.2 [d]		0.91		0.09
Met-Phe-Gly [b]	0.5	302	1.86	1.92	3.47	0.061	−14.1	8.5	7.1	0.54	0.41	0.05
		338	1.85	1.93	3.54	0.083	−14.3	8.4	6.7	0.53	0.37	0.10
		354	1.90	1.99	3.56	0.085	−14.4	8.3	6.9	0.52	0.39	0.09

[a] Downfield from internal t-butyl alcohol. [b] Recorded at 220 MHz; other spectra at 100 MHz. [c] $\frac{1}{2}(\delta_A + \delta_B)$. [d] $(J_{AX} + J_{BX})$. [e] $(a + b)$.

TABLE 2

^{1}H N.m.r. parameters and rotamer populations of phenylalanyl-containing peptides in basic solution

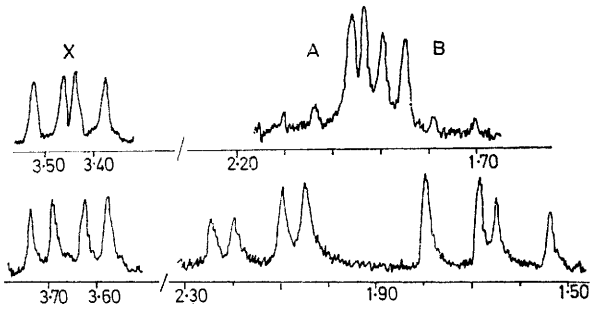
Peptide	pD	T/K	Chemical shifts [a] δ_B	δ_A	δ_X	$\delta_A - \delta_B$	Coupling constants J/Hz J_{AB}	J_{BX}	J_{AX}	Rotamer populations a	b	c
Leu-Phe	12.7	302	1.74	2.02	3.30	0.272	−13.9	8.7	5.0	0.55	0.22	0.23
		313	1.72	2.00	3.27	0.274	−14.2	8.8	5.1	0.56	0.23	0.21
		354	1.76	2.03	3.32	0.268	−14.3	8.5	5.3	0.54	0.25	0.21
Val-Phe	12.6	302	1.73	1.98	3.30	0.256	−14.1	9.0	5.1	0.58	0.23	0.19
		354	1.77	2.02	3.34	0.257	−14.4	8.8	5.1	0.56	0.23	0.21
His-Phe	13.1	302	1.70	1.89	3.19	0.198	−13.9	8.3	5.1	0.52	0.23	0.25
		317	1.72	1.92	3.21	0.200	−14.0	8.2	5.1	0.51	0.23	0.26
		354	1.78	1.99	3.29	0.210	−14.3	8.4	5.3	0.53	0.25	0.22
Met-Phe	12.6	302	1.69	2.00	3.31	0.316	−14.0	9.2	4.8	0.60	0.20	0.20
		311	1.72	2.04	3.30	0.317	−14.3	9.3	5.1	0.61	0.23	0.16
		354	1.75	2.05	3.33	0.300	−14.4	8.9	5.1	0.57	0.23	0.20
Tyr-Phe	12.5	302	1.73	1.89	3.23	0.156	−13.7	7.7	5.2	0.46	0.24	0.30
		317	1.74	1.91	3.28	0.167	−13.8	7.9	5.0	0.48	0.22	0.30
		354	1.79	1.98	3.31	0.188	−14.0	8.1	5.3	0.50	0.25	0.25
Phe-Val	13.3	302	1.74	1.77	2.46	0.127	−13.8	7.4	5.7	0.44	0.28	0.28
		328	1.68	1.83	2.50	0.149	−14.2	7.6	5.7	0.45	0.28	0.27
		350	1.70	1.87	2.54	0.164	−14.2	7.5	5.6	0.45	0.27	0.28
Phe-Leu	13.0	302	1.68	1.74	2.44	0.066	−13.8	6.6	5.7	0.36	0.28	0.36
		323	1.71	1.78	2.49	0.070	−14.2	7.3	5.5	0.43	0.26	0.31
		352	1.72	1.82	2.50	0.095	−14.2	7.3	5.7	0.43	0.28	0.29
Gly-Phe-Ala [b]	12.9	204	1.72	1.96	3.39	0.241	−13.8	9.3	5.3	0.61	0.25	0.14
		306	1.73	1.96	3.39	0.233	−14.0	9.1	5.2	0.59	0.24	0.17
		334	1.74	1.96	3.40	0.218	−14.1	9.0	5.5	0.58	0.26	0.16
Met-Phe-Gly	12.6	280	1.72	2.07	3.55	0.345	−13.8	10.5	5.2	0.72	0.24	0.04
		323	1.76	2.08	3.56	0.313	−14.2	10.1	5.2	0.68	0.24	0.08
Gly-Phe-Phe [b]	13.0	297	1.56	1.82	3.30	0.252	−14.0	9.4	5.4	0.62	0.25	0.13
Central Phe		308	1.57	1.82	3.31	0.245	−14.0	9.3	5.4	0.61	0.25	0.14
		297	1.69	1.93	3.18	0.237	−13.8	8.0	5.0	0.49	0.22	0.29
Terminal Phe		308	1.70	1.93	3.19	0.233	−13.8	8.0	4.9	0.49	0.21	0.30

[a] Downfield from internal t-butyl alcohol. [b] Recorded at 220 MHz; other spectra at 100 MHz.

ature to 348 K has no significant effect on the rotamer populations.

Table 2 lists the n.m.r. parameters for some phenylalanyl-containing di- and tri-peptides in basic solution. The corresponding chemical shifts, δ_A, δ_B, and δ_X, for the X-Phe dipeptides extend over a range of *ca.* 0.1 p.p.m., while the coupling constants indicate that all rotamers are appreciably populated, with (I) predominant. However, populations *a* are appreciably different for the dipeptides Met-Phe and Tyr-Phe. At 302 K $a = 0.60$ for Met-Phe and 0.46 for Tyr-Phe; these differences are accompanied by a corresponding change in $\delta_A - \delta_B$. For X-Phe dipeptides the population of rotamer (III) increases in acid over that in base, mainly at the expense of (II). For Phe-Leu, the rotamer populations become more disparate as temperature increases from 302 to 352 K; this is paralleled by an increase of $\delta_A - \delta_B$. From similar results for phenylalanine [17] and other aromatic amino-acids,[18] Cavanaugh concluded that the rotamer energies were temperature-dependent. He suggested changes in solute–solute and solute–solvent interactions as the cause, but these could be very complicated for the present dipeptides owing to the presence of both hydrophobic and hydrophilic groups. For the central residue of Gly-Phe-Ala, Gly-Phe-Phe, and Met-Phe-Gly, rotamer (I) is most populated. Rotamer (III), with the phenyl ring *gauche* to the peptide bonds, is appreciably populated (*ca.* 0.15) in Gly-Phe-Ala and Gly-Phe-Phe but only sparsely populated (0.04) in Met-Phe-Gly. The Phe residues in Gly-Phe-Phe were assigned by analogy with the vicinal coupling constants of Gly-Phe-Ala and from the results for the *C*-terminal residue of Phe-Phe in base (pD 11.5), with ($J_{AX} + J_{BX}$) 13 Hz and δ_X 3.20. Comparison of the rotamer populations for Gly-Phe-Ala and Met-Phe-Gly in acidic and basic solution shows rotamer (I) to be more and rotamer (II) less favoured; rotamer (III) is similarly populated under both conditions. Since a change from base to acid should cause downfield shifts, albeit small for the central residue, it is evident that variations in δ_A, δ_B, and δ_X reflect changes in conformations; this is apparent for $\delta_A - \delta_B$ of Met-Phe-Gly. All X-Phe di- and tri-peptides in basic solution have a larger value of J_{BX} than the 7.6 Hz for the phenylalanyl anion reported by Bartle *et al.*[1] Consequently, the population of the *trans*- rotamer (I) increases as the *N*-terminal amino group forms a peptide bond. Conversely, the formation of a *C*-terminal peptide bond as in Phe-Val has little effect on the population of rotamer (I). In acidic solution, J_{BX} for phenylalanine is 7.2 Hz [19] and the measured values for X-Phe dipeptides and X-Phe-Y tripeptides show that rotamer (I) increases in population.

Side-chain Rotations of Tyrosyl Residues in Di- and Tri-peptides.—The n.m.r. parameters and derived rotamer populations for some tyrosine-containing peptides in acidic solution are contained in Table 3. D-Leu-Tyr apart, all the X-Tyr dipeptides (X = Gly, Ala, Val, L-Leu, Trp, and Phe) have similar vicinal coupling constants and chemical shifts. The largest differences within the group occur for Trp-Tyr and L-Leu-Tyr; the shift differences are *ca.* 0.2 p.p.m., while the population of rotamer (I) is 0.49 and 0.59, respectively. In general, all three rotamers are appreciably populated, as observed for the similar X-Phe dipeptides (Table 1). The differences between the Tyr ABX spectra of the two Leu-Tyr diastereoisomers (Figure) are of particular interest. For



100 MHz ^{1}H Tyr ABX n.m.r. spectra of L-Leu-Tyr (upper) and D-Leu-Tyr (lower). Chemical shifts are in p.p.m. downfield from t-butyl alcohol

the DL-isomer the *trans*-rotamer is more favoured than for the LL-isomer, witness the respective values of J_{BX} and $\delta_A - \delta_B$. Increase of temperature causes a small reduction of the *a* population for both dipeptides and a decrease in $\delta_A - \delta_B$ for the DL-isomer.

The corresponding chemical shifts of the side-chain Leu resonances of the two diastereoisomers are significantly different; in particular, the composite C*H*–CH_2 absorption of the DL-isomer is 0.3 p.p.m. to high field of that in the LL-isomer. In view of similar results [20] for these peptides in trifluoroacetic acid, it is likely that these protons are closer to the face of the tyrosine ring in the DL- than in the LL-isomer. The upfield shift is consistent with a position having cylindrical co-ordinates *z ca.* 4 Å and ρ *ca.* 2—3 Å with respect to the shielding ring current of tyrosine.[21] For D-Leu-Tyr a molecular model with a rigid *trans* planar peptide $^+NH_3$ group remote from the peptide bond and a dihedral angle of *ca.* 180° for the Tyr N–C$^\alpha$ bond (J_{NH-CH} 9 Hz; see Table 5) suggests that this represents an energetically feasible average conformation. If so, the DL-isomer would have a more compact structure than the LL-isomer.

^{1}H N.m.r. parameters and rotamer populations from the deceptively simple 100 MHz ABX spectra of Tyr-Gly and Tyr-Val closely resemble those of Phe-X dipeptides. The vicinal coupling constants for the two tripeptides indicate more disparate populations for Val-Tyr-Val with a five-line ABX spectrum than for Gly-Tyr-Gly with a 12-line spectrum. Analogous results were

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obtained for some phenylalanyl-containing tripeptides; the problem of extracting rotamer populations from deceptively simple spectra has been discussed.[16]

Among the tyrosyl-containing peptides in basic solution (Table 4), Trp-Tyr apart, rotamer (I) predominates for all dipeptides but rotamers (II) and (III) are expense of (III). For Trp-Tyr, the Tyr methylene proton at higher field has the smaller coupling constant. This phenomenon has been observed previously [16] for some amino-acids but, without stereospecific monodeuteriation of the methylene group, it is difficult to distinguish unequivocally between the A and B protons

TABLE 3

^{1}H N.m.r. parameters and derived fractional rotamer populations for tyrosine-containing peptides in acidic solution

			Chemical shifts [a]				Coupling constants J/Hz			Rotamer populations		
Peptide	pD	T/K	δ_B	δ_A	δ_X	$\delta_A - \delta_B$	J_{AB}	J_{BX}	J_{AX}	*a*	*b*	*c*
Gly-Tyr	0.8	301	1.71	1.90	3.43	0.184	−14.1	8.5	5.6	0.54	0.27	0.19
		326	1.73	1.91	3.45	0.176	−14.1	8.7	5.9	0.55	0.30	0.15
		353	1.78	1.95	3.52	0.172	−14.7	8.5	6.1	0.54	0.32	0.14
Ala-Tyr	0.7	301	1.76	1.91	3.38	0.150	−14.2	8.8	5.7	0.56	0.28	0.16
		328	1.77	1.91	3.47	0.145	−14.3	8.6	5.8	0.55	0.29	0.16
		354	1.81	1.96	3.48	0.144	−14.7	8.7	6.0	0.55	0.31	0.16
Val-Tyr	0.8	301	1.76	1.87	3.38	0.110	−14.3	8.6	6.2	0.55	0.33	0.12
		327	1.84	1.95	3.49	0.118	−14.5	8.6	6.2	0.55	0.33	0.12
		353	1.85	1.97	3.51	0.120	−14.7	8.3	6.2	0.52	0.33	0.15
L-Leu-Tyr	0.9	302	1.85	1.95	3.43	0.106	−14.5	9.1	5.9	0.59	0.30	0.11
		317	1.86	1.96	3.45	0.108	−14.4	9.1	6.2	0.59	0.33	0.08
		358	1.90	2.01	3.50	0.105	−14.6	8.7	6.1	0.55	0.32	0.13
D-Leu-Tyr	0.8	302	1.59	2.09	3.55	0.504	−14.4	11.6	4.6	0.82	0.18	
		326	1.67	2.13	3.65	0.457	−14.7	11.4	4.8	0.80	0.20	
		354	1.72	2.12	3.64	0.408	−15.0	11.1	4.9	0.77	0.21	0.02
Trp-Tyr	0.8	302	1.59	1.72	3.21	0.127	−14.1	8.0	6.0	0.49	0.31	0.20
		326	1.64	1.78	3.29	0.141	−14.5	8.0	6.2	0.49	0.33	0.18
Phe-Tyr	1.0	302	1.71	1.82	3.32	0.108	−14.6	8.3	6.2	0.52	0.33	0.15
		332	1.74	1.85	3.38	0.113	−14.6	8.3	6.2	0.52	0.33	0.15
Trp-Gly	1.4	303	1.94		3.06			14.0		0.80		0.20
Tyr-Val	0.8	301	1.86		3.02			14.4		0.84		0.16
		327	1.89		3.06			14.4		0.84		0.16
		353	1.96		3.15			14.4		0.84		0.16
Gly-Tyr-Gly [b]	0.9	296	1.67	1.83	3.39	0.156	−13.9	8.4	6.4	0.53	0.35	0.12
		303	1.70	1.85	3.42	0.149	−13.8	8.6	6.3	0.55	0.34	0.11
		323	1.72	1.86	3.45	0.145	−13.7	8.3	6.4	0.52	0.35	0.13
		348	1.74	1.89	3.47	0.145	−13.6	8.2	6.4	0.51	0.35	0.14
Val-Tyr-Val [b]	0.8	297	1.75		3.43			1.55		0.94		0.06

[a] Downfield from internal t-butanol. [b] Recorded at 220 MHz; other spectra at 100 MHz.

TABLE 4

^{1}H N.m.r. parameters and derived fractional rotamer populations for tyrosine-containing peptides in basic solution

			Chemical shifts [a]				Coupling constants J/Hz			Rotamer populations		
Peptide	pD	T/K	δ_B	δ_A	δ_X	$\delta_A - \delta_B$	J_{AB}	J_{BX}	J_{AX}	*a*	*b*	*c*
L-Leu-Tyr	13.1	302	1.58	1.78	3.13	0.197	−14.2	8.1	5.0	0.50	0.22	0.28
		323	1.63	1.82	3.19	0.190	−14.3	8.1	5.1	0.50	0.23	0.27
		354	1.62	1.80	3.18	0.186	−14.5	8.0	5.4	0.49	0.25	0.26
D-Leu-Tyr	12.6	302	1.50	1.81	3.16	0.307	−14.1	9.4	4.6	0.62	0.15	0.23
		323	1.53	1.79	3.15	0.263	−14.1	8.9	4.7	0.57	0.19	0.24
		354	1.59	1.82	3.20	0.230	−14.6	8.6	5.0	0.55	0.22	0.23
Trp-Tyr [b]	12.3	302	1.30	1.48	3.05	0.174	−14.1	5.4	6.3	0.25	0.34	0.41
		358	1.55		3.17			12.4		0.65		0.35
Val-Tyr	12.6	302	1.57	1.77	3.16	0.205	−14.2	8.4	5.0	0.53	0.22	0.25
		317	1.61	1.81	3.21	0.203	−14.4	8.3	5.3	0.52	0.25	0.23
		354	1.61	1.81	3.22	0.194	−14.6	8.2	5.4	0.51	0.25	0.24
Tyr-Phe	12.5	302	1.28	1.50	2.22	0.221	−14.0	7.6	5.2	0.45	0.24	0.31
		317	1.26	1.51	2.22	0.251	−13.9	7.9	5.2	0.48	0.24	0.28
		354	1.25	1.56	2.27	0.311	−14.3	8.3	5.3	0.52	0.25	0.23
Tyr-Val	12.7	302	1.48	1.69	2.42	0.215	−13.9	7.7	5.0	0.46	0.22	0.32
		326	1.45	1.69	2.38	0.234	−14.0	7.8	5.0	0.47	0.22	0.31
		354	1.48	1.24	2.42	0.256	−14.3	8.0	5.2	0.49	0.24	0.27

[a] Downfield from internal t-butyl alcohol. [b] Recorded at 220 MHz; other spectra at 100 MHz.

appreciably populated. The $C_\alpha H$ chemical shift is remarkably consistent; however, the vicinal coupling constants show variations, particularly with increase of temperature. For L-Leu-Tyr, D-Leu-Tyr, and Val-Tyr, rotamer (II) increases, chiefly at the expense of (I), while for Tyr-Phe and Tyr-Val rotamer (I) increases at the and hence to determine the sign of $\delta_A - \delta_B$. At 302 K, rotamer (III) predominates but its population decreases as the temperature is raised to 358 K. The spectrum at 358 K is of special interest in that, in contrast to the spectra of the tripeptides Val-Tyr-Val and Met-Phe-Met, it yields $J_{AX} + J_{BX}$ 12.4 Hz as $\delta_A - \delta_B$ approaches

zero; this suggests equal rotamer populations. Simultaneous changes occur in the aromatic region of the spectrum. At 302 K, $\delta_A - \delta_B$ of the Tyr AA'BB' spin system is small and a singlet is observed at 220 MHz; however, at 358 K, it has increased to 0.35 p.p.m. and the spectrum has its more common symmetrical 'four-line' appearance. Comparison of the Tyr chemical shifts with those of other X-Tyr dipeptides reveals that the ring protons *meta* to the hydroxy-group have moved *ca.* 0.4 p.p.m. downfield. For dipeptides containing aromatic amino-acids, interactions between adjacent residues have been indicated [22] with consequent changes in the chemical shifts of ring protons. However, protons situated above or below the plane of the highly anisotropic Trp ring [21] are generally shifted upfield. A molecular model of Trp-Tyr constructed with a planar *trans*-peptide bond, NH_2 group most remote from the peptide bond, and a *trans* proton at the Tyr N–C_α bond, suggests that steric interactions will prevent the two rings approaching more closely than 6 Å. At these separations, ring-current effects would be small and changes in the average orientation of the Tyr ring to the anisotropic peptide bond may contribute to the variation in chemical shift of the ring protons. Trp-Tyr apart, J_{BX} increases relative to the amino-acid value of 7.2 Hz, so that rotamer (I) is favoured.

Rotational Isomerism of the N–C_α *Bond.*—For small peptides in solution, the observed coupling constant J_{NH-CH} (Table 5) is expected to be a weighted ϕ average of the distribution of conformations. From the calculated feasible ϕ values and the angular dependence of J_{NH-CH}, values of 7.9 and 6.8 Hz have been proposed.[23] In view of the similarity of the value for the alanine residue, the conformation of the N–C_α alanine bond could involve a distribution of conformations as described by conformational-energy diagrams such as Figures 3—5 in ref. 24; in these, 70% of the allowed conformations are in the region with ϕ from −30 to −160° and ψ from −60 to +180°. For other peptides, J_{NH-CH} is *ca.* 7.4 Hz; relative to the alanine conformation, this means an increased contribution from conformations with the two hydrogens *trans*. For the valyl residue of Val-Tyr-Val, $J_{NH-CH} = 8.5$ Hz, consistent with 8.9 [25] and 8.5 Hz reported previously,[26] and indicates a further contribution from *trans*-hydrogens. Steric interactions between side-chains and the adjacent peptide bond severely restrict acceptable values of ϕ and ψ,[27] *e.g.* to ϕ between −80 and −140° for the valyl residue; the Bystrov graph [28] suggests J_{NH-CH} 8—10 Hz, consistent with the 8.5 Hz measured. For alanine and glycine, a wider range of ϕ values is allowed and J_{NH-CH} is found to be smaller. For the diasteroisomers of Leu-Tyr, J_{NH-CH} of the DL-isomer indicates a greater *trans*-hydrogen contribution than for the LL-isomer

TABLE 5

^{1}H N.m.r. parameters of amide resonances

			Central residue		*C*-Terminal residue	
Peptide	pH	*T*/K	δ(NH) [a]	J_{NH-CH}/Hz	δ(NH) [a]	J_{NH-CH}/Hz
Gly-Phe	1.0	294			7.5	7.5
Gly-Phe-Ala	1.5	297	7.30	7.3	7.2	6.5
Met-Phe-Gly	0.7	302	7.5	7.4	7.1	11.0
Gly-Tyr	1.0	294			7.4	7.5
Val-Tyr-Val	0.6	302	7.4	7.2	6.9	8.5
Gly-Tyr-Gly	1.0	297	7.30	7.4	7.16	11.0
D-Leu-Tyr	0.5	302			7.2	9.0
L-Leu-Tyr	0.5	302			7.2	7.3

[a] Downfield from t-butyl alcohol.

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