Conformational Features of Benzoyl *N*-Alkylated Amino-acids (*N*-Alkylated Benzamido-acids) determined by Nuclear Magnetic Resonance Spectroscopy

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cis–trans Rotational isomerism about the benzoyl amide bond has been detected in a series of *N*-alkylated benzamido-acids. The proportions of *cis–trans* rotamers appear to be different for *N*-methylvaline and *N*-methylisoleucine when compared with other imino-acids studied. A singlet peak for the aromatic protons in the ^{1}H n.m.r. spectra of a number of derivatives, shows that the *ortho*-protons are not deshielded and that conjugation between the benzene ring and the amide group is reduced because of the steric environment created by *N*-alkylation of the amide. U.v. measurements also confirm this phenomenon.

A NUMBER of n.m.r. studies have been reported on the *cis–trans* rotational isomerism about the amide bonds of proline-containing peptides [1] and acylated cyclic imino-acids.[2] The acyclic imino-acids have not been so extensively investigated,[3] although they represent

[1] R. Deslauriers, I. C. P. Smith, and M. Rothe in ' Peptides; Chemistry Structure and Biology,' eds. R. Walter and J. Meienhofer, Ann Arbor Science Publishers, Chicago, 1975, p. 91; I. C. P. Smith, R. Deslauriers, and K. Shamburg, *ibid.*, p. 97; R. Garner and W. B. Watkins, *Chem. Comm.*, 1969, 386; C. M. Deber, F. A. Bovey, J. E. Carver, and E. R. Blout, *J. Amer. Chem. Soc.*, 1970, **92**, 6191; V. Madison and J. Schellmann, *Biopolymers*, 1970, **9**, 511; H. Okabayoshi and T. Isemura, *Bull. Chem. Soc., Japan*, 1970, **43**, 359; O. Oster, E. Breitmaier, and W. Voelter in ' N.M.R. Spectroscopy of Nuclei Other Than Protons,' ed. T. Axenrod and G. A. Webb, Wiley, New York, 1974, p. 233.

[2] H. L. Maia, K. G. Orrell, and H. N. Rydon, *J.C.S. Perkin II*, 1976, 761; W. A. Thomas and M. K. Williams, *J.C.S. Chem. Comm.*, 1972, 788.

[3] M. Goodman and N. S. Choi, Peptides: Proceedings of the Ninth European Peptide Symposium, 1968, p. 1; B. Liberek, K. Steporowska, and E. Jereczek, *Chem. and Ind.*, 1970, 1263; S. L. Portnova, V. F. Bystrov, T. A. Balashova, V. T. Ivanov, and Yu. A. Ovchinnikov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1970, 825; M. Goodman, F. Chen, and C-Y. Lee, *J. Amer. Chem. Soc.*, 1974, **96**, 1479.

important constituents of peptide antibiotics.[4] Synthetic studies [5] using *N*-methylamino-acids have shown that contrary to expectation, the *N*-methylamino-acids give rise to more racemisation than would be expected from the accepted mechanism.[6] With the availability of an n.m.r. method [7] for quantitative analysis of diastereoisomers, it became necessary to investigate the n.m.r. spectra of *N*-alkylated benzamido-acids to study their application in the detection of racemisation. During this study interesting features were seen in the spectra and this paper describes our attempt at their explanation.

Benzoyl derivatives of the acyclic *N*-methylamino-acids were prepared in two ways, using (a) the conventional Schotten–Baumann benzoylation of the *N*-methylamino-acid and (b) the *N*-methylation of the benzoylated amino-acid using methyl iodide and sodium hydride.[8] Route (a) was used for the preparation of the benzoyl derivatives of the cyclic imino-acids.

The 100 MHz ^{1}H n.m.r. spectrum of benzoyl-*N*-methylvaline is reproduced in Figure 1 and represents an

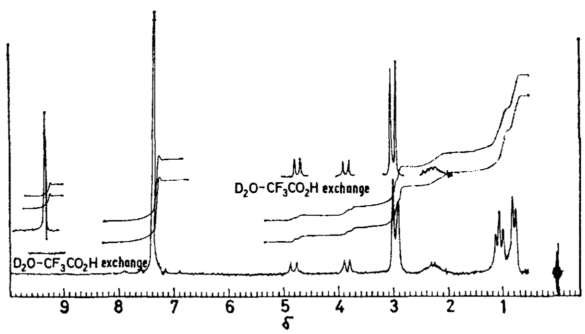
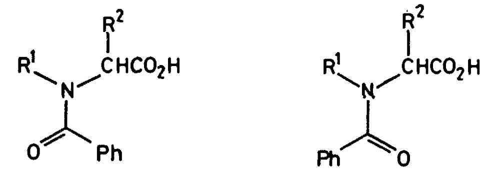


FIGURE 1 100 MHz ^{1}H N.m.r. spectrum of benzoyl-*N*-methylvaline

ambient temperature spectrum fairly typical of the acyclic imino-acids studied. Chemical shifts for other acyclic benzoylated imino-acids are summarised in Table 1, together with the results of variable temperature studies. Table 2 summarises the details of spectra obtained for the cyclic imino-acids, while Table 3 lists ^{13}C shifts and assignments.

In all the examples studied, except *N*-benzoylazetidine-2-carboxylic acid, separate signals representing the *cis*-form (A) and the *trans*-form (B) could be identified in the ^{1}H n.m.r. spectra. Two well separated signals were present for the α-proton in (A) and (B) while the two signals associated with the *N*-alkyl substituent being closer together at the slow exchange limit, required lower temperatures generally to resolve the separate forms. Previous assignments [1,2] of signals to individual rotamers have assumed that the *trans*-form would be predominant and thus could be assigned the stronger signals. Solvent shifts and the use of the Paulsen



(A) (B)

model [9] for anisotropy of the amide carbonyl have also served as a means of confirming these assignments. However the use of the former argument implying a predominance of *trans*-rotamer is not so applicable in our examples since the conformers are often almost equal in population. Moreover, the large anisotropic effect exerted by the phenyl group overwhelms the smaller effect of the amide carbonyl, and the Paulsen considerations do not apply. A study of the nuclear Overhauser effect has also proved fruitful in identifying conformers,[10] but when attempted on our compounds, no definite peak assignment was possible. A discussion of these abortive attempts is considered later.

Our approach to peak assignment for the *cis–trans* rotamers, is based on an analysis of the ^{13}C spectra of the aliphatic ring carbons in the cyclic imino acid derivatives. The ring carbon resonances of C_β and C_γ show a characteristic *cis–trans* pattern,[11] and in a wide variety of proline derivatives. We consider the β- and γ-carbons to be far enough away from the benzene ring to afford the same conformational pattern as for the aliphatic acyl derivatives. In Figure 2 a schematic representation of the ^{13}C spectrum of benzoylproline is shown, and on comparison with the previously known pattern,[11] it is seen that the *trans* is the predominant form in benzoylproline. Similarly the pattern of peaks for the β- and δ-carbons in benzoylpipecolic acid (Figure 2), suggests a predominance of the *trans*-form. On extrapolation of these assignments to the C_α and NCH_2 carbon signals, it seems plausible to assign the weaker downfield signal for C_α to the *cis*-conformer and the stronger to the *trans*. Similarly the NCH_2 carbon signals show a pattern in which the stronger *trans*-signal is downfield of the *cis*. Extrapolation of this information to the ^{1}H n.m.r. spectra indicates that the *trans* α-proton is downfield of the *cis* while the *trans*-NCH_2 protons are upfield of *cis* (in $CDCl_3$ solution). This situation implies that in the proline and pipecolic acid derivatives the shielding effect of the benzoyl group is consistent with the data on aliphatic acyl

[4] C. H. Hassall and W. A. Thomas, *Chem. in Brit.*, 1971, **7**, 145; B. W. Bycroft and C. M. Wels, in 'Amino-acids, Peptides and Proteins,' ed. R. C. Sheppard, Chemical Society, 1976, vol. 8, ch, 4, p. 310; and corresponding chapters in previous volumes of this series; C. H. Hassall, in 'Peptides; Chemistry Structure, and Biology,' eds. R. Walter and J. Meienhofer, Ann Arbor Science Publishers, Chicago, 1975, p. 891.

[5] J. R. McDermott and N. L. Benoiton, *Canad. J. Chem.*, 1973, **51**, 2562.

[6] M. W. Williams and G. T. Young, *J. Chem. Soc.*, 1964, 3701.

[7] J. S. Davies, R. J. Thomas, and M. K. Williams, *J.C.S. Chem. Comm.*, 1975, 76.

[8] J. R. McDermott and N. L. Benoiton, *Canad. J. Chem.*, 1973, **51**, 1915.

[9] H. Paulsen and K. Todt, *Angew. Chem. Internat. Edn.*, 1966, **5**, 89; *Chem. Ber.*, 1967, **100**, 3385, 3397.

[10] B. P. Roques, S. Combrisson, and R. Wasylishen, *Tetrahedron*, 1976, **32**, 1517.

[11] C. Grathwohl and K. Wuthrich, *Biopolymers*, 1976, **15**, 2025; W. A. Thomas and M. K. Williams, *J.C.S. Chem. Comm.*, 1972, 994.

substituents. This is in agreement with recent observations [10,12] on the aroyl derivatives of proline. We also confirm that in benzoylproline the chemical shifts of the methine α-proton are relatively insensitive to solvent changes.[12]

It does not necessarily follow, however, that we should see the same shielding effects in the ^{1}H n.m.r. spectra of acyclic *N*-methylamino-acids, since the relative conformation of the protons near the benzoyl group would be altered. On the other hand it is plausible that the methyl carbon of a *N*-methyl group in *e.g.* *N*-benzoyl-*N*-methylalanine would have the same relative position to the benzoyl group as the ε-carbon in benzoylpipecolic acid, and similarly the α-carbons would be expected to

TABLE 1

Proton chemical shifts (δ 100 MHz) of acyclic amino-acid derivatives

Compound * (in $CDCl_3$)	Temp. (°C)	C_6H_5	α-C–H	N–CH_3	Other protons
BzMeGly	Room	7.43(s)	4.28 3.94	3.05br(s)	8.91(OH)
	−45°	7.47(s), 7.39(s)	4.32 3.93	3.07(s), 3.10(s)	
Bz-DL-MeAla	Room	7.39(s)	5.22(q), 4.38(q)	2.90br(s)	1.26—1.58(m, CHCH_3), 11.45 (OH)
	0°	7.43(s), 7.37(s)	5.31(q), 4.39(q)	2.93(s), 2.90(s)	1.52(d), 1.36,(d, CHCH_3)
	+48°	7.38(s)	5.30—4.30br	2.88br	1.40(d CHCH_3)
Bz-DL-Ala	Room	7.80(d), 7.88(d) *ortho*; 7.48—7.34 *meta* and *para*	4.59 (quintet)		1.49(d, CHCH_3)
Bz-L-MeLeu	Room	7.41(s)	5.25(t), 4.33(t)	2.93(s), 2.87(s)	1.45—1.95(m, CH_2C*H*), 1.01(d), 0.83(d), 0.59[m, C*H*(CH_3)$_2$]
	−10°	7.44(s), 7.39(s)	5.32(t), 4.32(t)	2.94(s), 2.90(s)	1.42—2.0(m, CH_2C*H*), 1.02(d), 0.84(d), 0.56[m, CH(CH_3)$_2$], 10.0 (OH)
	+50°	7.40(s)	5.20br, 4.30br	2.90br	1.76br (CH_2C*H*), 0.97br [CH(CH_3)$_2$], 9.06 (OH)
Bz-L-Val	Room	7.71(d), 7.78(d) *ortho*; 7.37 *meta* and *para*	4.69, 4.79(dd)		2.29(m, C*H*Me_2), 0.99[d, CH(CH_3)$_2$], 7.08 (NH)
Bz-L-MeVal	Room	7.41(s)	4.78(d), 3.85(d)	2.99(s), 2.92(s)	2.26(m, β-CH), 1.07(t), 0.81[d, CH-(CH_3)$_2$], 11.36 (OH)
	−20°	7.45(s), 7.43(s)	4.84 (d), 3.79(d)	2.99(s), 2.93(s)	2.23(m, β-CH), 1.07(t), 0.78[d, CH-(CH_3)$_2$]
Bz-D-NorVal	Room	7.82(d), 7.74(d) *ortho*; 7.49—7.35 *meta* and *para*	4.79(m)		1.86(m, β-CH_2), 1.42(m, CH_2CH_2), 0.92(t, CH_3), 6.97(NH), 7.2(OH)
Bz-D-MeNorVal	Room	7.40br(s)	5.23(m), 4.33(m)	2.94(s), 2.88(s)	1.84(m, β-CH_2), 1.54—1.24(m, CH_2CH_2), 1.10—0.78(m, –CH_3)
	0°	7.44(s), 7.38(s)	5.26, 5.15(dd), 4.29, 4.21(dd)	2.96(s), 2.91(s)	2.07—1.61(m, β-CH_2), 1.55—1.19(m, CH_2CH_2), 1.01(t), 0.79(t, CH_3)
Bz-D-Ile	Room	7.93(d), 7.85(d) *ortho*; 7.53—7.29 *meta* and *para*	4.51(d), 4.43(d)		2.01(m, β-CH), 1.43(m, γ-CH_2), 1.11—0.81(CH_3CH–CH_2CH_3), 8.13(d, NH)
Bz-D-MeIle	Room	7.41(s)	4.93(d), 3.99(d)	3.00(s), 2.95(s)	2.03(β-C*H*), 1.39(γ-CH_2), 1.13—0.69 (CH_3CHCH_2CH_3)
	−14°	7.45(s), (7.41(s)	4.95(d), 3.93(d)	3.05(s), 2.97(s)	1.99(β-C*H*), 1.37(γ-CH_2), 1.11—0.67 (CH_3CHCH_2CH_3)
BzNMe_2	Room	7.37(s)		3.03br(s)	
	−5°	7.36(s)		3.05(s), 2.91(s)	

* Nomenclature as defined by IUPAC Rules, *i.e.* L-MeVal represents L-*N*-methylvaline.

TABLE 2

Proton chemical shifts (δ 100 MHz) of cyclic imino-acid derivatives

Compound (in $CDCl_3$)	*T*/°C	C_6H_5		α-CH	NCH_2	Other protons
Bz-DL-pipecolic acid *	28	7.40 (s)		5.54 (m) (major), 4.42 (minor)	4.66 (d, H_{eq}) (minor), 3.68 (d, H_{eq}) (major) 3.21 (m, H_{ax}) (minor), 2.81 (H_{ax}) (major)	2.41—1.20 (m, β,γ,δ)
Bz-L-proline *	28	7.59(d) 7.53(d) *ortho*	7.49—7.31 *meta* and *para*	4.78 (d), 4.70 (d) (major)	3.52 (m) (minor)	2.40—1.80 (m, β,γ)
				4.32 (m) (minor)	3.72 (m) (major)	10.5 (OH)
Bz-L-azetidine-2-carboxylic acid *	28	7.7br (s) 7.63 (d) *ortho*	7.51—7.37 *meta* and *para*	5.14 (t)	4.32 (m)	2.68, 2.56 (dd, β-CH_2)
Bzpiperidine	Room	7.6—7.35br (m)			3.62 (m), 3.4 (m)	1.96—1.80 (CH_2CH_2)

* Assignments based on comparisons with other imino-acid derivatives, W. A. Thomas and M. K. Williams, published and unpublished results.

[12] H. Nishihara, K. Nishihara, T. Uefuji, and N. Sakota, *Bull. Chem. Soc. Japan*, 1975, **48**, 553.

TABLE 3

^{13}C N.m.r. shifts (p.p.m.) for benzoyl derivatives at ambient temperature (proton-decoupled spectra)

Compound (in $CDCl_3$)	Carbonyl groups COOH	Carbonyl groups N–CO	Aromatic carbons	Cα	$NCH_3(CH_2)$	Cβ	Cγ	Cδ
BzMeGly	173.28	171.44	134.52, 128.43, 127.12	53.16	38.78			
	173.03	170.85	126.62	49.20	34.58			
BzAla ($CDCl_3$–[$^2H_6$]DMSO)	174.66	167.05	134.01, 131.37, 128.22, 127.34	48.47		17.63		
BzMeAla	174.69	172.99	135.15, 130.0, 128.48, 127.11	57.23	34.09	15.26		
	173.28		126.58	52.71	29.33	15.17		
BzNorVal ($CDCl_3$–[$^2H_6$]DMSO)	174.30	167.22	134.26, 131.28, 128.16, 127.46	52.56		33.59	19.05	13.66
BzMeNorVal	175.21	173.53	135.49, 130.09, 128.57, 127.14	61.47	34.32	30.99	19.61	13.57
	174.05		126.85	56.80	29.24	30.21	19.14	
BzVal	174.95	168.55	133.67, 131.98, 128.62, 127.28	57.76		31.34	19.0	
							17.88	
BzMeVal	174.01	172.85	135.03, 129.88, 128.48, 127.20	67.48	34.56	27.72	19.91, 19.43	
	173.48	171.52	126.91	62.70	29.48	27.14	19.03, 18.85	
BzMeLeu	175.30	173.43	135.45, 129.97, 128.48, 127.05	60.03	34.18	37.97	25.21	23.23
	173.95			55.07	29.53	36.92	24.40	21.39
BzMeIle	173.74	172.75	135.29, 130.21, 128.49, 127.18	66.44	35.15	34.04	25.34, 15.86	10.95
			129.84	62.41	29.57	33.13	24.93, 15.74	
BzPro	174.42	170.78	135.51, 130.33, 128.20, 127.09	62.0	50.27	32.34	25.18	
				59.6	45.47	28.74	21.28	
BzPipe	175.51	172.30	135.32, 129.88, 128.49, 127.03	58.37	46.03	27.38	21.10	25.39
	174.55		126.53	52.45	40.13	26.50		24.69
BzAz		171.82	132.13, 128.71, 127.96	62.46	51.84	19.96		

Az = Azetidine-2-carboxylic acid, Pipe = pipecolic acid.

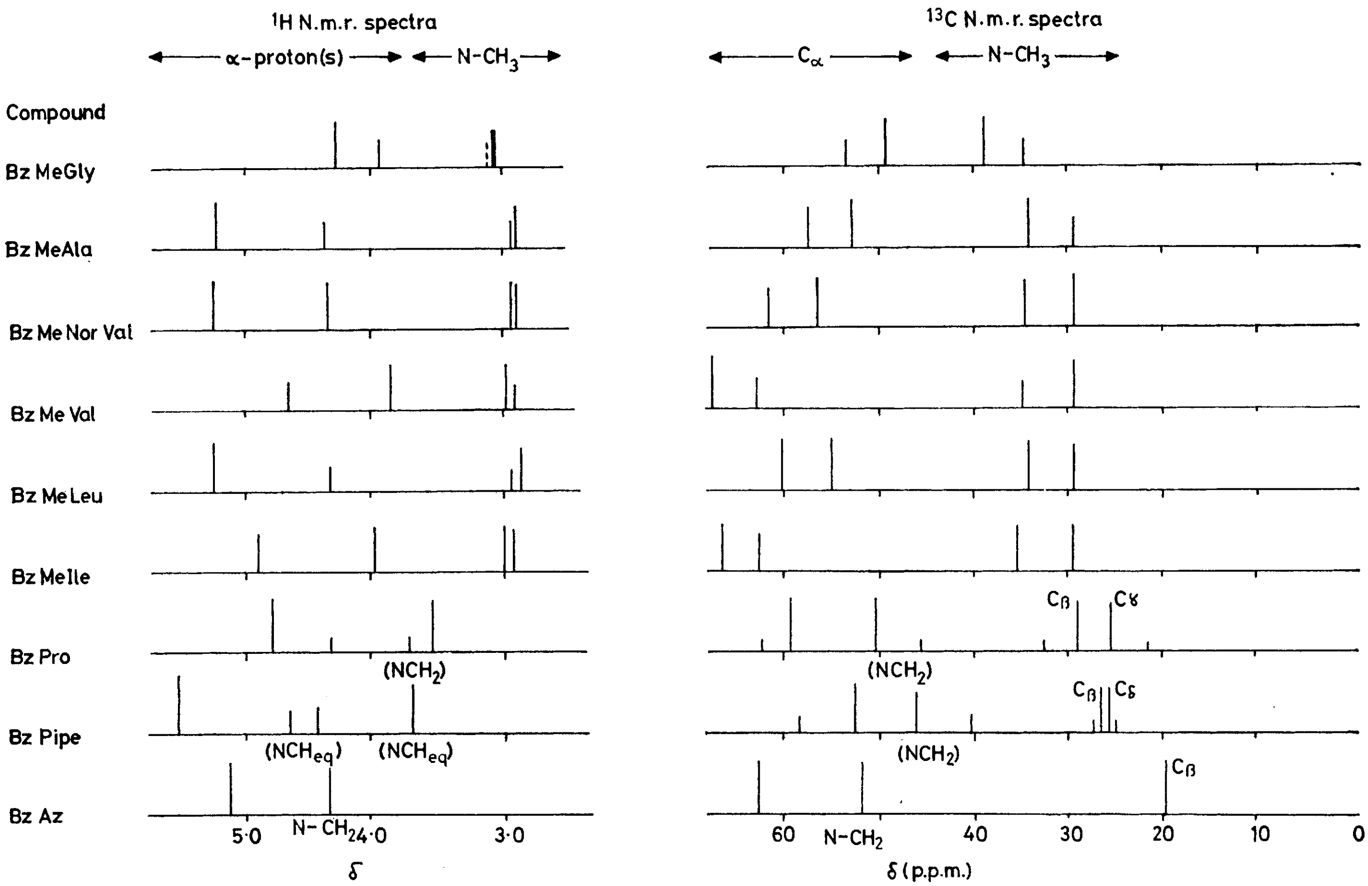


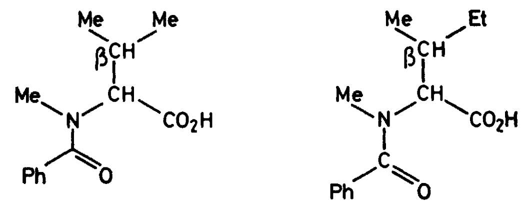
FIGURE 2 Diagrammatic representation of peak intensities showing *cis–trans* isomers

bear the same relationship. On the basis of this comparison with the ^{13}C n.m.r. spectra of the cyclic compounds it can be deduced from the ^{13}C n.m.r. section of Figure 2, that the benzoyl derivatives of MeGly and MeAla exist predominantly in the *trans*-form. Without placing too much emphasis on the detailed intensities of carbon resonance signals, it can also be seen that MeNorVal and MeLeu, seem to exist almost equally in *cis*- and *trans*-conformations, while MeIle and MeVal show signals assigned to the *cis*-form as having the highest intensities.

Absolute intensities of carbon resonances in the ^{13}C n.m.r. spectra will be dependent upon nuclear Overhauser enhancement and spin lattice relaxation effects, so that a categorical judgement of the *cis* : *trans* ratio is not advisable on the above evidence alone. However a similar trend can be seen in the ^{1}H n.m.r. spectra (Table 1 and Figure 2). Where MeGly, MeAla, and MeLeu derivatives show a similar *cis* : *trans* intensity pattern to proline and pipecolic acid, MeVal and to a lesser extent MeIle show the opposite effect. BzMeNorVal seems to favour an almost equal distribution of conformers. By making this comparison between the cyclic and acyclic imino-acids, we therefore assign the downfield signal for the α-proton to the *trans*-rotamer and the upfield signal to the *cis*-form. Signals for the NCH_3 protons as expected show the opposite relationship. These assignments would therefore suggest that the benzoyl group has a different effect on the neighbouring protons to that reported for acetyl derivatives of MeGly [13] and MeAla.[14] It is also significant that the benzoyl group gives rise to much higher amounts of the *cis*-form in non-polar solvents, when compared with related acetyl derivatives.[15]

By averaging out intensity ratios measured from both ^{13}C and ^{1}H n.m.r. spectra, the results shown in Table 4 were obtained. It may be argued that the trend towards a predominance of the *cis*-form in MeIle is not very significant. However on cooling the sample to −15 °C, the ratios *cis* : *trans* become 68 : 32 and 60 : 40 for MeVal and MeIle respectively based on integration of proton signals. Another consequence of lowering the temperature was an improved separation of the *cis*- and *trans*-NCH_3 signals, whose coalescence temperature is very near ambient in some cases.

The predominance of the *cis*-form in BzMeVal and BzMeIle is somewhat surprising. As can be seen in structures (1) and (2) the only significant difference between these molecules and the other examples of similar side-chain size is the increased substitution on the β-carbon. On steric considerations alone therefore, it would be much more feasible for these molecules to be *trans*, with the aromatic ring as far away as possible from the amino-acid side-chain. However it can be argued that in non-polar solvents, that hydrogen bonding [16] between the carboxy group and the benzoyl carbonyl would tend to stabilise the *trans*-form, in such a way as to bring the side-chain into close proximity with the *N*-methyl group. Therefore increasing the



trans-(1) *trans*-(2)

NCH_3–alkyl side-chain interaction by β-substitution, may induce the molecule to rotate in such a way as to remove the hydrogen bonding interaction, and in so doing give more scope for the aryl ring to move into the *cis*-position.

Support for this hypothesis comes from the observation that addition of $[^{2}H_6]$DMSO to a $CDCl_3$ solution of BzMeVal changes the *cis* : *trans* rotamer ratio from 60 : 40 to 50 : 50. Moreover the methyl ester of BzMeVal, which cannot give rise to hydrogen bonding exists as a 50 : 50 mixture of *cis* : *trans* rotamers in $CDCl_3$ solution.

TABLE 4

cis : *trans* Ratios of benzoylated amino-acids in $CDCl_3$ at ambient temperature

	MeGly	MeAla	MeVal	MeNorVal	MeLeu	MeIle	Pro	Pipe	Az
cis : *trans* (^{1}H n.m.r.)	42 : 58	47 : 53	57 : 43	51 : 49	46 : 54	54 : 46	(25 : 75) *	(35 : 65) *	0 : 100
cis : *trans* (^{13}C n.m.r.)	38 : 62	43 : 57	63 : 37	48 : 52	49 : 51	55 : 45	(12 : 88)	(22 : 78)	0 : 100
Average (approx.)	40 : 60	45 : 55	60 : 40	50 : 50	48 : 52	55 : 45	20 : 80	25 : 75	0 : 100

* Peak overlap and separation of conformers into axial and equatorial forms prevents precise measurement.

The results obtained for the benzoylazetidine derivative also deserve comment. In the ^{13}C n.m.r. spectrum only one resonance signal was seen for each of the C_α, NCH_2, and carboxy carbons. With a more complex splitting pattern for the protons in the ^{1}H n.m.r. spectrum it is more difficult to identify different conformers, but at ambient temperature there is no hint of separation of peaks into *cis*- and *trans*-signals. Three interpretations of these results are possible, that the molecule exists totally in one conformation, that the chemical shifts are the same in *cis*- and *trans*-forms, or that rotation is too fast to be detected on the n.m.r. time scale. We favour the first of these interpretations, since separate investigations [16] have shown that in $CDCl_3$, *N*-acetylazetidine-2-carboxylic acid is 90% *trans*-conformer.

An unusual feature in the ^{1}H n.m.r. spectra of the benzoyl derivatives of the acyclic imino-acids studied is the appearance of the aromatic protons as a 5H singlet at δ 7.4 at both 60 and 100 MHz, *i.e.* the *ortho*-protons are not deshielded relative to the others. Benzoyl derivatives of the non-*N*-methylated analogues all show *ortho*-deshielding effects. We interpret this effect to be due to the benzene ring rotating out of the plane of the amide

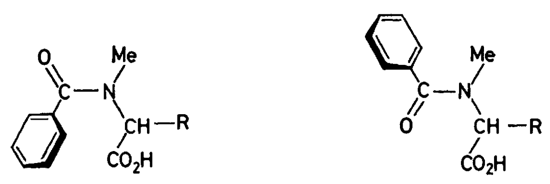
[13] F. A. Bovey, J. J. Ryan, and F. P. Hood, *Macromolecules*, 1968, **1**, 305.

[14] M. Goodman and M. Fried, *J. Amer. Chem. Soc.*, 1967, **89**, 1264.

[15] V. T. Ivanov, P. V. Kostetskii, T. A. Balashova, S. L. Portnova, E. S. Efremov, and Yu. A. Ovchinnikov, *Khim Prirod Soedinenii*, 1973, 339.

[16] W. A. Thomas and M. K. Williams, *Org. Magnetic Resonance*, 1972, **4**, 145.

group as a result of *N*-methylation. Space-filling models of *NN*-dimethylbenzamide (which also shows a singlet for the aromatic protons) show that with the phenyl ring and amide group coplanar there is very unfavourable interaction with the *cis*-methyl group. It is therefore likely that interference is relieved by rotation about the Ph–CO bond. The out-of-plane aromatic ring takes the *ortho*-protons out of the range of the deshielding cone of the carbonyl group. Table 5 summarises the u.v. maxima for the compounds and the shift to shorter wavelengths resulting from the *N*-methylation of the benzoyl amide bond supports the hypothesis of a reduction in conjugation. An analogous trend to the n.m.r. results can be seen in the u.v. maxima of the cyclic imino-acid derivatives. U.v. data [17] of substituted benzamides and a recent n.m.r. study [18] on *NN*-diethylbenzamide also indicate a similar trend. An *X*-ray study [19] on *p*-bromo-*NN*-dimethylbenzamide showed that the plane of the aromatic ring formed an angle of 45° with the plane of the carbonyl group and the Me_2N group is turned through 9° with respect to the plane of the carbonyl group. We conclude therefore by analogy that the conformation of the acyclic *N*-methylated amino-acid derivatives would be based on the structures illustrated in Figure 3.

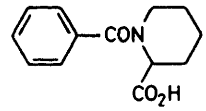


cis *trans*

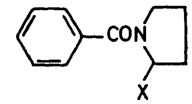
FIGURE 3 Conformation of acyclic *N*-methylated amino-acid derivatives

When the ^1H n.m.r. spectra were measured at low temperatures the aromatic protons separate into two singlets, which appear in similar integral ratios to the *cis–trans* rotamers identified from the α-proton and *N*-alkyl proton patterns.

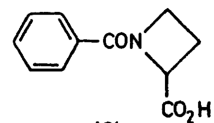
The aromatic protons also appear as a singlet in the ^1H n.m.r. spectrum of *N*-benzoylpipecolic acid (3). Thus when the nitrogen atom is part of a six-membered ring there is still sufficient steric interaction to push the benzene ring out of plane. However deshielding of the *ortho*-protons can be seen in benzoylpyrrolidine (4), *N*-benzoylproline (5), and to a greater extent in *N*-benzoylazetidinecarboxylic acid (6). We conclude



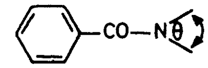
(3)



(4) X = H
(5) X = CO_2H



(6)



(7)

therefore that the angle θ in (7) is important in deciding whether the aromatic ring can be accommodated in the plane of the amide group, or whether non-bonded interactions with the nitrogen substituents force it to prefer the out-of-plane conformation.

TABLE 5

Bz-amino-acid	$\lambda_{max.}$(EtOH)/nm	ε	Bz-*N*-alkylated amino-acid	$\lambda_{max.}$(EtOH)/nm	ε
BzVal	236	8 500	BzMeVal	211	7 200
BzNorVal	232	8 500	BzMeNorVal	209	8 100
BzIle	232	7 500	BzMeIle	211	7 200
BzLeu	235	7 500	BzMeLeu	211	8 100
BzAla	230	9 400	BzMeAla	211	7 500
BzGly	231	7 800	BzMeGly	211	7 700
			BzPipe	212	8 000
			BzPro	220	6 700
			BzAz	234	6 600

Returning, finally to the lack of success in the nuclear Overhauser effect (NOE) studies. In the knowledge that the aromatic ring is pushed out of plane of the amide group, it is probably not surprising that when either the NCH_3 protons or the aromatic protons were irradiated in *NN*-dimethylbenzamide, and benzoyl-*N*-methylvaline, there were no corresponding enhancement in the other resonance signals. Even when these studies were carried out at low temperatures [10] to reduce fast interconversion of rotamers no effect could be detected. Thus it seems that the out of plane conformation would take the aromatic protons too far away from the NCH_3 group for an NOE enhancement to be detected.

EXPERIMENTAL

^1H N.m.r. spectra were determined at 100 MHz on a Varian HA-100 instrument. Variable temperature experiments were also carried out on this instrument using the V-4341 temperature control unit. Methanol was used as standard to calibrate the low temperature ranges. ^{13}C N.m.r. spectra were obtained on a Varian XL-100 instrument at 25.2 MHz. Tetramethylsilane was used as internal standard for all spectra. Optical rotations were measured on a Perkin-Elmer 141 automatic polarimeter and u.v. measurements on a Perkin-Elmer 402 spectrophotometer.

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Preparation of N-*Benzoyl Derivatives.—Method A. Benzoylation of amino-acids.*[20] The amino-acid (0.005 mol) in 2M-NaOH (2.2 ml) was cooled in an ice-bath and treated with benzoyl chloride (0.0055 mol) in small portions alternating with additions of 2M-NaOH (2.2 ml). Vigorous shaking and cooling of solution was maintained throughout, and it was kept alkaline by addition of more alkali. After 30 min the solution was acidified, but in contrast to the amino-acids, the crude benzamido-acids separated as gums. The supernatant liquid was removed, the gum washed with ice–water, and any contaminating benzoic acid removed by boiling with tetrachloromethane (benzoic acid crystallised out). The mother liquor yielded the benzamido-acids which were further purified by recrystallisation.

Method B. Methylation of benzamido-acids.[8] The *N*-benzamido-acid (0.01 mol; prepared from the corresponding amino-acid using the conditions above) and iodomethane (5 ml, 0.08 mol) in tetrahydrofuran (30 ml) were cooled to 0 °C in a flask protected from moisture. Sodium hydride (B.D.H.; 80% dispersion in oil; 1.32 g) was added cautiously with stirring, and with the separation of solid the suspension became viscous, but stirring was continued for 24 h. The sodium hydroxide formed was destroyed by addition of ethyl acetate, followed by dropwise addition of water. The solution was evaporated to dryness and the oily residue partitioned between ether (30 ml) and water (100 ml). The ether layer was washed with aqueous $NaHCO_3$, and the combined extracts acidified. Extraction of the product into ethyl acetate, followed by washing with water and 5% sodium thiosulphate and drying ($MgSO_4$) gave the benzoyl derivatives as pale yellow oils which were purified by crystallisation: *N*-benzoylmethylglycine (sarcosine) had m.p. 104—107 °C (from ethyl acetate) (lit.,[21] 104—105 °C); *N*-benzoyl-*N*-methyl-DL-alanine, m.p. 128—131 °C (from ether) (lit.,[22] 129—130 °C); *N*-benzoyl-*N*-methyl-D-norvaline, m.p. 127—129 °C (from ether) (Found: C, 66.5; H, 7.3; N, 6.2. $C_{13}H_{17}NO_3$ requires C, 66.35; H, 7.3; N, 5.95%), $[\alpha]_D$ +14° (*c* 0.5 in methanol); *N*-benzoyl-*N*-methyl-L-valine, m.p. 85—86 °C (from ether) (Found: C, 66.45; H, 7.3; N, 6.28), $[\alpha]_D^{25}$ −135° (*c* 1.0 in methanol); *N*-benzoyl-*N*-methyl-L-leucine, m.p. 132—134 °C (lit.,[23] 135—137 °C), $[\alpha]_D^{25}$ −40° (*c* 0.25 in methanol) (lit.,[23] −55° in DMF); *N*-benzoyl-*N*-methyl-L-isoleucine, m.p. 109—112 °C (Found: C, 67.65; H, 7.65; N, 5.4. $C_{14}H_{19}NO_3$ requires C, 67.45; H, 7.7; N, 5.6%), $[\alpha]_D^{25}$ −100° (*c* 0.4 in methanol); *N*-benzoyl-L-proline, m.p. 154—156 °C (from ethanol–water) (lit.,[24] 158—159°) (Found: C, 66.15; H, 6.2; N, 6.25. Calc. for $C_{12}H_{13}NO_3$: C, 65.75; H, 6.0; N, 6.4%), $[\alpha]_D^{25}$ −97° (*c* 1 in methanol) (lit.,[24] −100° in DMF); *N*-benzoyl-DL-pipecolic acid, m.p. 124—126 °C (from toluene–ethyl acetate) (lit.,[25] 125—127 °C); *N*-benzoyl-L-azetidine-2-carboxylic acid, m.p. 118—121 °C (from ether) (Found: C, 64.6; H, 5.68; N, 6.75. $C_{11}H_{11}NO_3$ requires C, 64.4; H, 5.4; N, 6.85%), $[\alpha]_D^{25}$ −196° (*c* 0.5 in methanol). *NN*-Dimethylbenzamide, m.p. 42—44 °C (lit.,[26] 42—43 °C), and *N*-benzoylpyrrolidine[27] were synthesised from benzoyl chloride and the corresponding amine using standard conditions.

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