Natural Abundance Nitrogen-15 Nuclear Magnetic Resonance Spectroscopy. Medium Effects on the Nitrogen-15 Chemical Shifts of Small Peptides

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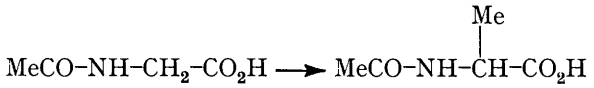
Medium effects upon the ^{15}N chemical shifts of some small peptides are reported, to allow the relative importance of solvent, pH, and sequence effects to be delineated. A change in the pH of a solution of carnosine (β-alanylhistidine) from 0.4 to 11.0 causes the His ^{15}N resonance to shift downfield by 8.5 p.p.m. Changing the solvent from dimethyl sulphoxide (DMSO) to trifluoroacetic acid (TFA) causes downfield shifts of *ca.* 4 p.p.m. for the peptide nitrogen resonances of some *N*-acetyldipeptides, and explains why the ^{15}N shift observed for polyglycine in TFA solution is to low field of that predicted earlier for a glycine residue of a peptide in DMSO solution. The magnitude of these pH and solvent effects is such that they may mask any sequence effects upon peptide ^{15}N shifts, which are reported to be 1—4 p.p.m. for dipeptides in aqueous solution.

MAGNETIC resonance spectra of the ^{15}N nucleus are being used increasingly in the study of peptides.[1-9] Two important features of these studies are the conclusions of Roberts and his co-workers [5] and conclusions from this laboratory.[4] The former group found that the chemical shifts of the peptide ^{15}N nuclei in simple dipeptides (aqueous solutions; pH range 5.0—6.1) were slightly sensitive to the nature of both *C*-terminal and *N*-terminal units. Thus it is formally possible that sequence information may be obtained by ^{15}N n.m.r., a non-destructive method. Our studies,[4] however, indicated that there was no detectable sequence information from ^{15}N chemical shifts of *N*-acetyldipeptides, at least in dimethyl sulphoxide (DMSO) solution. Accordingly we have undertaken a study of medium effects (including both solvent and pH effects) upon peptide ^{15}N chemical shifts for some simple model systems, to clarify the situation.

Assignment of ^{15}N Resonances.—The first stage in the investigation of peptides by ^{15}N n.m.r. is the assignment of the resonances to specific amino-acid units. We shall assume initially that sequence effects upon the ^{15}N shifts are small and that in the absence of any differential effects upon the amide nitrogens in the peptide (caused by solvation, hydrogen bonding, or conformation changes), the relative ^{15}N shifts of the amide nitrogens are controlled mainly by the nature of the amino-acid unit itself.

Previously published ^{15}N chemical shifts of amino-acids,[1] *N*-formyl amino-acids,[4,10] *N*-acetyl amino-acids,[4] and amino-acid methyl ester hydrochlorides [10,11] allow estimation of the effect of substitution at the amino-acid α-carbon atom upon the ^{15}N shifts. In the manner previously adopted [1,11] we assume an additivity relationship for these substituent effects similar to that of Grant and Paul [12] for ^{13}C chemical shifts. The approach is exemplified by the difference in ^{15}N shifts for the *N*-acetyl derivatives of glycine and alanine,[4] which are 89.4 and 104.4 p.p.m. respectively. This change is taken to be due to a methyl substituent which is β to the nitrogen atom in the latter:



* Data from ref. 4; 1M-solutions in DMSO; chemical shifts to low field of $^{15}NH_4{}^{15}NO_3$ (see footnote *a*, Table 2).

Therefore, $\Delta\beta_N = +15.0$ p.p.m. (with the convention such that the ^{15}N resonance from the alanine derivative is at lower field). Extension of this treatment to valine, leucine, and isoleucine derivatives, in which saturated carbon substituents are introduced at positions γ and δ to nitrogen, yields $\Delta\gamma_N = -3.8$ and $\Delta\delta_N = +0.6$ p.p.m. The data [4] for the phenylalanine, serine, and cysteine derivatives give the γ effects for the substituents Ph, OH, SH. A similar analysis may be performed for the other amino-acid derivatives mentioned above. The substituent effects are collected in Table 1, together with appropriate ^{13}C parameters where available from the literature. The correlation between ^{15}N and ^{13}C chemical shift substituent parameters is good, in both magnitude and direction (upfield or downfield). Similar comparisons between ^{15}N and ^{13}C chemical shift substituent parameters have been made by Lichter,[13] and Warren and Roberts [14] for ^{15}N shifts in saturated amines; by Gibbons and his co-workers [1] for ^{15}N-enriched amino-acids; and by Pregosin *et al.*[10,11] for some of the amino-acid methyl ester hydrochlorides.

With the substituent parameters shown in Table 1, it is

[1] J. A. Sogn, W. A. Gibbons, and E. W. Randall, *Biochemistry*, 1973, **12**, 2100.
[2] T. Suzuki, T. Yamaguchi, and M. Imanari, *Tetrahedron Letters*, 1974, 1809.
[3] G. E. Hawkes, W. M. Litchman, and E. W. Randall, *J. Magnetic Resonance*, 1975, **19**, 255.
[4] G. E. Hawkes, E. W. Randall, and C. H. Bradley, *Nature*, 1975, **257**, 767.
[5] T. B. Posner, V. Markowski, P. Loftus, and J. D. Roberts, *J.C.S. Chem. Comm.*, 1975, 769.
[6] C. S. Irving and A. Lapidot, *J. Amer. Chem. Soc.*, 1975, **97**, 5945.
[7] M. Llinas, W. J. Horsley, and M. P. Klein, submitted to *Proc. Nat. Acad. Sci., U.S.A.*
[8] D. Gust, R. B. Moon, and J. D. Roberts, *Proc. Nat. Acad. Sci., U.S.A.*, 1975, **72**, 4696.
[9] K. Wüthrich, 'N.M.R. in Biological Research: Peptides and Proteins,' North-Holland, Amsterdam and New York, 1976.
[10] A. I. White, Ph.D. Thesis, University of London, 1972.
[11] P. S. Pregosin, E. W. Randall, and A. I. White, *Chem. Comm.*, 1971, 1602.
[12] D. M. Grant and E. G. Paul, *J. Amer. Chem. Soc.*, 1964, **86**, 2985.
[13] R. L. Lichter in 'Determination of Organic Structures by Physical Methods,' vol. 4, eds. F. C. Nachod and J. J. Zuckerman, Academic Press, New York and London, 1971.
[14] J. P. Warren and J. D. Roberts, *J. Phys. Chem.*, 1974, **78**, 2507.

then possible to predict relative ^{15}N shifts for the amide nitrogens in a peptide chain by using the following approximations. (i) Sequence effects may be neglected to a first-order approximation. In contrast to our observations, Roberts and co-workers [5] found sequence shifts in

TABLE 1

^{15}N Chemical shift substituent parameters [a] for some amino-acid derivatives

	Methyl ester Hydrochlorides [b]	*N*-Formyl [b]	*N*-Acetyl [b]	[^{15}N]Amino-acids [c]	^{13}C
$\Delta\beta_N$(C)	+13.0	+15.0	+15.0	+12.9	+9.4 [d]
$\Delta\gamma_N$(C)	−2.3	−3.5	−3.8	−3.5	−2.5 [d]
$\Delta\delta_N$(C)	+0.6	+0.6	+0.5	+1.2	+0.4 [d]
$\Delta\gamma_N$(Imid) [e]	−0.8				
$\Delta\gamma_N$(Ph)	−2.1	−4.3	−3.5	−3.7	−3.0 [f]
$\Delta\gamma_N$(OH)	−6.2				−6.0 [d]
$\Delta\gamma_N$(SH)	−3.6		−5.4		
$\Delta\gamma_N$(CO_2Me)	−3.7				−2.8 [d]
$\Delta\gamma_N$(CO_2H)				−3.2	−2.7 [d]
$\Delta\delta_N$(SMe)	+0.5	−0.5			

[a] Values in p.p.m.; positive values indicate downfield effect. [b] Values extracted from data in ref. 4. [c] Data from ref. 1. [d] Data from T. Pehk and E. Lippmaa, *Org. Magnetic Resonance*, 1971, **3**, 679. [e] The imidazole substituent; value obtained from the histidine derivative. [f] Data from J. B. Stothers, 'Carbon-13 NMR Spectroscopy,' Academic Press, New York and London, 1972.

dipeptides, but these were 4 p.p.m. or less. This assumption may not be valid to the second order, and is the subject of continuing studies. (ii) There is no large differential effect at the amide nitrogens. The main published work on this point concerns the cyclic peptides Gramicidin S [3,4] and alumichrome.[7] Each of these peptides may have one or two N–H bonds projected towards the centre of the ring (with specific intramolecular C=O · · · H–N hydrogen bonding [15,16]), with the remainder more subject to intermolecular or solvent interactions. Differential solvent effects of 4 p.p.m. in Gramicidin S and 5 p.p.m. in alumichrome have been observed. For non-cyclic systems we report here (see below) that a differential solvent effect on the amide nitrogen chemical shifts of some *N*-acetyldipeptides has been observed for the solvents DMSO and trifluoroacetic acid (TFA). (iii) There are no differential conformation effects at the amide nitrogens. In this context we have observed [4,10] a ^{15}N shift difference of 1.6 p.p.m. between the *cis*- and *trans*-forms of *N*-formylproline in DMSO solution.

pH *Dependence of the* ^{15}N *Chemical Shifts.*—The model compounds diglycine (p*K* 3.14 and 8.25) and triglycine (p*K* 3.23 and 8.09) were selected as the starting point for this study. The ^{15}N chemical shifts are summarised in Table 2. From the data on diglycine it can be seen that protonation of the carboxylate group (variation of pH in the range 7.4—0.5) causes the peptide nitrogen resonance to shift upfield by 6.2 ± 0.6 p.p.m. There is little effect upon the shift of the amino-group (p*K* 8.25) in this pH range. The peptide nitrogen resonances of triglycine (which are closely spaced at pH 0.5), are well resolved at pH 8.0. At this higher pH the assignment of the resonances is readily made by comparison with the data for diglycine:

$$\overset{+}{H_3N}-CH_2-CO-NH-CH_2-CO-NH-CH_2-CO_2^-$$
$$88.6 \qquad 94.8 \text{ p.p.m.}$$

For the protonated form of triglycine (pH 0.5) the peptide nitrogen assignments are not so sure, however. Since the peptide nitrogen resonance of diglycine moves upfield by 6.2 ± 0.6 p.p.m. upon protonation of the carboxylate function, then at pH 0.5 the peptide nitrogen resonance of the *C*-terminal glycine residue is to be expected at 94.8 − (6.2 ± 0.6) p.p.m., *i.e.* in the region 88.0—89.2 p.p.m. Thus our assignment at pH 0.5 is:

$$\overset{+}{H_3N}-CH_2-CO-NH-CH_2-CO-NH-CH_2-CO_2H$$
$$87.5 \qquad 88.9 \text{ p.p.m.}$$

The data in Table 2 show that the amino-resonances of di- and tri-glycine are observed only at the more acidic pH values. This phenomenon will be shown to occur

TABLE 2

^{15}N Chemical shifts [a] for di- and tri-glycine

pH	Peptide		Amino	
0.5	88.3		6.7	Diglycine
5.8	94.5		6.2	Diglycine
7.4	94.5		*b*	Diglycine
0.5	87.5	88.9	6.7	Triglycine
8.0	88.6	94.8	*b*	Triglycine

[a] In p.p.m. (±0.3) downfield from $^{15}NH_4^+$ resonance of 5M-$^{15}NH_4{}^{15}NO_3$ in 2N-HNO_3 (see ref. 20 for this choice). The ^{15}N reference employed by Roberts and his co-workers [5,8] is external 1M-$D^{15}NO_3$, whereas our external reference is as above. Comparison of our data on diglycine with those of Roberts and his co-workers [5] (pH 5.8) indicates that the two scales are related by the equation $\delta(^{15}NH_4^+) = (355.0 \pm 0.1) - \delta(D^{15}NO_3)$. Positive shifts relative to ammonium ion are downfield; those from nitric acid are upfield. [b] Resonances not observed (see text).

(see below) with carnosine (a dipeptide) and glutathione (a tripeptide). Other workers [17,18] have noted that the intensity of the proton-decoupled ^{15}N resonance in glycine is strongly pH dependent. They have attributed this either [17] to scalar relaxation of ^{15}N induced by chemical exchange modulation of the ^{15}N–^1H scalar coupling, or [18] to spin–rotation relaxation for ^{15}N. Each of these relaxation mechanisms should affect the ^{15}N–{^1H} nuclear Overhauser enhancement (NOE) and thus the ^{15}N signal intensity. However, Irving and Lapidot [6] have recently demonstrated that these pH dependent ^{15}N signal intensities in glycine and in diglycine are certainly due to the presence of paramagnetic impurities (mainly Cu^{2+}). Such contamination of the samples was presumably the reason for our difficulties in observing amino-resonances in aqueous solution at all but acidic pH values. However,

[15] A. Stern, W. A. Gibbons, and L. C. Craig, *Proc. Nat. Acad. Sci., U.S.A.*, 1968, **61**, 734.
[16] T. P. Pitner and D. W. Urry, *J. Amer. Chem. Soc.*, 1972, **94**, 1399.
[17] R. A. Cooper, R. L. Lichter, and J. D. Roberts, *J. Amer. Chem. Soc.*, 1973, **95**, 3724.
[18] T. K. Leipert and J. H. Noggle, *J. Amer. Chem. Soc.*, 1975, **97**, 269.

we have not experienced any unusual difficulty in observing amide nitrogen resonances at any of the pH values employed.

The natural abundance ^{15}N spectra (chemical shifts summarised in Table 3) of glutathione (γ-glutamylcysteinylglycine) in both oxidised and reduced (pK 2.12, 3.59, 8.75, and 9.65) forms illustrate the effect of remote as well as proximate ionisation upon peptide nitrogen chemical shifts (see Figure 1). The pH dependence of

TABLE 3

^{15}N Chemical shifts [a] for glutathione

Oxidised form (–S–S–)				Reduced form (SH)			
pH	Cys	Gly	Glu-NH_3^+	pH	Cys	Gly	Glu-NH_3^+
0.4	102.2	90.7	19.5	0.4	102.2	90.7	19.0
4.0	102.8	94.2	21.1	2.4	102.2	91.3	19.5
7.3	102.8	96.1	21.4	7.5	103.0	96.6	*b*
12.0	103.6	96.6	*b*	12.5	106.5	96.6	12.0

[a] See footnote *a*, Table 2. [b] Resonance not observed (see text).

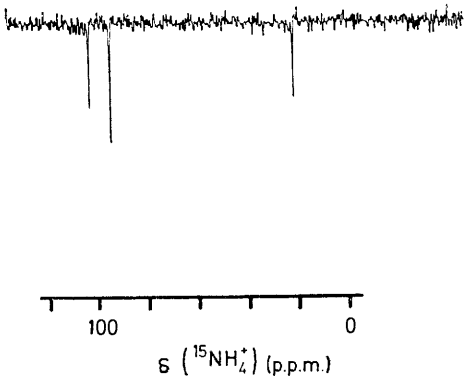
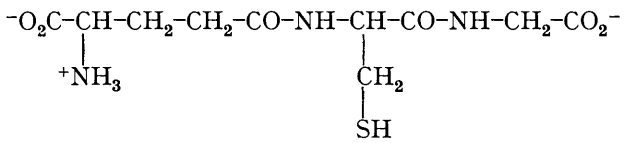


FIGURE 1 Natural abundance ^{15}N spectrum of glutathione (oxidised form) at pH 4.0, ^1H noise decoupled; *ca.* 200 000 free induction decays accumulated in 22 h. The signals are inverted by the ^{15}N-{^1H} NOE. Sample concentration 1.1M in H_2O

the ^{13}C chemical shifts has been studied recently by Feeney *et al.*[19] The two lower field resonances are due to the cysteine (or cystine) and glycine amide nitrogens,



Glutathione (reduced form)

and the higher field of these two is due to glycine. This may be readily deduced from the substituent parameters in Table 1. The third, highest field, resonance is due to the Glu α-$\overset{+}{N}H_3$.

Comparison of the chemical shifts in Table 3 for the oxidised (dithio) and reduced (SH) forms at pH 0.4 shows that replacement of the SH grouping by the –S–S– bridge has no effect upon the amide nitrogen shifts. This is in accord with the similarity in ^{15}N chemical shifts of cysteine and cystine methyl ester hydrochlorides.[4,10] In the oxidised form of glutathione there are three ionisable groups, Glu α-CO_2H, Glu α-NH_3^+, and Gly α-CO_2H. The Gly-^{15}N signal is moved strongly downfield (5.9 p.p.m.) by the ionisation of Gly-CO_2H to Gly-CO_2^-, whereas the cystine ^{15}N shift varies but little (1.4 p.p.m.) over the whole pH range 0.4—12. In the reduced form, glutathione has the additional ionisable group Cys-SH (pK 9.65). The effect of the ionisation of this group, Cys-SH to Cys-S^-, is to impart a downfield shift of 4.3 p.p.m. to the Cys ^{15}N resonance (see the data at pH 0.4 and 12.5). In addition this ionisation imparts a small upfield shift to the Gly ^{15}N resonance (*cf.* data at pH 7.5 and 12.5). That the ^{15}N shifts are downfield both for the Gly nitrogen for the Gly α-CO_2H ionisation and for the Cys nitrogen for the Cys-SH ionisation is not surprising, since each ionisation involves a proton at a position γ to the nitrogen of interest (*i.e.* is effectively a δ-substituent effect due to the proton). The Glu α-NH_3^+

resonance moves upfield by 7.0 p.p.m. upon deprotonation (data at pH 0.4 and 12.5), and this agrees in direction with the ^{15}N shift of 12.5 p.p.m. in glycine between pH 6.6 and 13.6 as reported by Roberts and his coworkers.[17]

The pH dependent ^{15}N chemical shifts of carnosine (β-alanylhistidine, pK 2.64, 6.87, and 9.51) are shown in Table 4. Over the pH range investigated (0.35—11) the

TABLE 4

^{15}N Chemical shifts [a] in carnosine

	Imidazole			
pH			Peptide	Amine
0.4	155.3	152.2	101.8	12.0
4.5	*b*	*b*	105.5	11.5
8.0	*b*	*b*	108.9	10.7
10.8	*b*	*b*	109.9	*b*
11.0	*b*	*b*	110.3	*b*

[a] See footnote *a*, Table 2. [b] Resonances not observed (see text).

amide ^{15}N resonance moves downfield by 8.5 p.p.m. with increasing pH. In addition to the effects of ionisation of the carboxyl-group, this shift will include a contribution from the deprotonation of the imidazole ring (pK 6.83). The imidazole substituent is γ to the amide nitrogen, the same relative position as the SH group is to the cysteine amide nitrogen in the reduced form of glutathione.

Here again at the higher pH values employed the amino-resonance was not observed, and indeed the imidazole nitrogen signals were only observed at the most acid pH (0.35).

Solvent Dependence of ^{15}N *Chemical Shifts.*—An important feature of solution studies on peptides is the helix- to-random-coil conformational conversion in certain homopolypeptides which may be induced by changes in the

[19] J. Feeney, P. Partington, and G. C. K. Roberts, *J. Magnetic Resonance*, 1974, **13**, 268.

solvent system. We have obtained the ^{15}N spectrum of polyglycine in TFA solution (random-coil form). The ^{1}H-decoupled natural abundance ^{15}N spectrum is shown in Figure 2. The single resonance peak is inverted by the

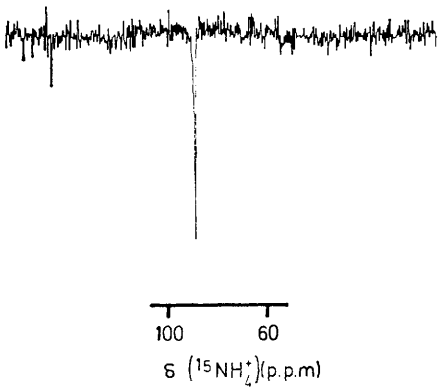
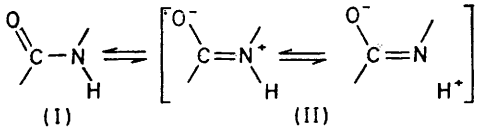


FIGURE 2 Natural abundance ^{15}N spectrum of polyglycine, ^{1}H noise decoupled; *ca.* 140 000 free induction decays accumulated in 16 h. The signal is inverted by the ^{15}N-{^{1}H} NOE. The sample was a solution of 125 mg of polyglycine in 1 ml of TFA

^{15}N-{^{1}H} NOE, which means [3,4] that the effective correlation time for reorientation of the amide ^{15}N–^{1}H bond vector is $\leqslant 6 \times 10^{-9}$ s. For longer correlation times an upright signal should be obtained. The chemical shift measured for this resonance was 88.3 p.p.m. In an earlier publication [4] we predicted the ^{15}N chemical shift of glycine in a peptide chain to be 84 ± 0.5 p.p.m. (in DMSO solution). For alumichrome, Llinas *et al.*[7] observed a shift of 3.7 p.p.m. to lower field for certain amide nitrogens on changing the solvent from DMSO to 2,2,2-trifluoroethanol, a protic acid solvent. This observation was ascribed to preferential stabilisation of structure (III) by the more acidic solvent. Our data on polyglycine are



entirely consistent with this, the glycine ^{15}N shift being *ca.* 4 p.p.m. to lower field in TFA solution in comparison with that predicted for DMSO solution.

To further investigate the effect of solvent upon amide nitrogen chemical shifts we have recorded the ^{15}N spectra of some acetyl amino-acids and small peptides (Table 5; see also Figure 3). The effect of changing solvent from DMSO to TFA is to shift the ^{15}N resonance strongly downfield for AcGly and AcLeu (9.9 and 11.5 p.p.m., respectively). In both the solvents DMSO and TFA the lower field resonance from AcLeuGly must be due to Leu. Both the Leu and Gly resonances shift downfield on changing from DMSO to TFA; by 10.5 (Leu) and 4.3 p.p.m. (Gly). This differential downfield shift for the acetylated nitrogen and the nitrogen of the *C*-terminal residue is maintained for AcGly^{1}Gly^{2}: 9.8 (Gly^{1}) and 3.8 p.p.m. (Gly^{2}). For AcGly^{1}Gly^{2}Gly^{3} the magnitudes of the solvent-induced shifts are 10.0 (Gly^{1}) and 3.3 p.p.m. (Gly^{2} and Gly^{3}). The ^{15}N shift here of Gly^{2} (87.3 p.p.m. in TFA) is very similar to that for polyglycine in TFA (88.3 p.p.m.).

TABLE 5

^{15}N Chemical shifts [a] for some *N*-acetyl amino-acids and peptides

Compound [b]	Solvent		
AcGly	TFA	99.3	
	DMSO [c]	89.4	
AcLeu	MeOH	103.6	
	TFA	113.2	
	DMSO [c]	101.7	
AcGly^{1}Gly^{2}	H_2O	92.9 (Gly^{1})	88.4
	TFA	98.9 (Gly^{1})	87.8
	DMSO [c]	89.1 (Gly^{1})	84.0
AcLeuGly	TFA	112.7 (Leu)	88.9
	DMSO [c]	102.2 (Leu)	84.6
AcGly^{1}Gly^{2}Gly^{3}	TFA	97.7 (Gly^{1})	87.3 (Gly^{2}, Gly^{3})
	DMSO [c]	89.7 (Gly^{1})	84.0 (Gly^{2}, Gly^{3})

[a] See footnote *a*, Table 2. [b] Concentrations 0.8—1.1M. [c] Values from ref. 4.

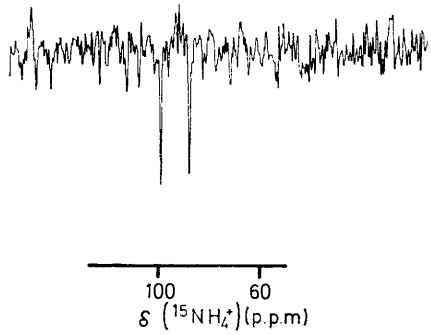


FIGURE 3 Natural abundance ^{15}N spectrum of *N*-acetylglycylglycine, ^{1}H noise decoupled; *ca.* 50 000 free induction decays accumulated in 5.5 h. The signals are inverted by the ^{15}N-{^{1}H} NOE. Sample concentration 1.1M in TFA

The ^{15}N shifts for the *C*-terminal Gly residues of AcLeuGly and AcGlyGly in DMSO solution are, within our error limits, the same, and do not reflect any sequence information. However, for the solutions in TFA we observe the *C*-terminal Gly ^{15}N signal at 1.5 p.p.m. to lower field for AcLeuGly. This shift difference is larger than our error limits (± 0.6 p.p.m. for the spectra compared) and must be due to the difference in the other amino-acid residue (AcLeu *cf.* AcGly). For the dipeptides LeuGly (pH 5.4) and GlyGly (pH 5.1) Roberts and his co-workers [5] found the amide ^{15}N signal to be 3.3 p.p.m. to lower field for the leucyl peptide. The origin of these sequence shifts is not clear, but it appears that they are solvent dependent.

Conclusion.—In favourable cases it is possible to assign the ^{15}N resonances from small open chain peptides to specific amino-acid residues by consideration of the substituent effects for the derivatives shown in Table 1. The range of ^{15}N shifts for these derivatives is 7 p.p.m. if we exclude glycine and proline, or 22 p.p.m. if we include these two. This range is expected to hold for the same amino-acid residues in peptides. It seems clear

that sequence effects upon the ^{15}N shifts will be small (0—4 p.p.m.) and dependent upon the solvent employed. The effect of changing the pH in the range 0—12 has been shown to influence the amide ^{15}N chemical shifts by as much as 8.5 p.p.m. for the His nitrogen of carnosine. Ionisations occurring at a centre very remote ($\geqslant$6 bonds) from the amide nitrogen of interest do not have a significant effect upon the ^{15}N shift as shown by the similarity of ^{15}N shifts for Gly^2 of $Gly^1Gly^2Gly^3OH$ (pH 0.5) and $Gly^1Gly^2Gly^3O^-$ (pH 8.0). Where such ionisation shifts do occur they may be significantly larger than the reported [5] sequence shifts. In addition these ionisation shifts may be larger than the substituent shifts which, to a first-order approximation, determine the ^{15}N chemical shift of a given amino-acid residue in a peptide chain. Thus considerable caution is required in assigning ^{15}N resonances from a peptide to specific residues.

The differential effects upon the ^{15}N chemical shifts of *N*-acetyl peptides produced by the solvent change from DMSO to TFA may assist in the assignment of the *N*-acetyl resonance.

EXPERIMENTAL

^{15}N Spectra were obtained with a Bruker HFX-13 n.m.r. spectrometer with a field strength of 2.141 T and a ^{15}N frequency of 9.12 MHz. The Fourier transform technique was used with a pulse angle of about 30° and no delay between pulses. The free induction decays were stored as 4 098 data points by using a Fabritek 1074 CAT instrument. Transformations were conducted with a PDP8/I computer with phase correction, but without exponential filtering on the free induction decay. Real, frequency domain spectra (5 kHz width) were produced in 2 048 data points.

Proton-noise decoupling was produced with the Bruker BSV 3B unit, and the Bruker ^2H time-shared lock unit was employed. Between 50 000 and 200 000 free induction decays were accumulated for each sample, since the samples contained only the natural abundance of ^{15}N; 10 mm o.d. tubes were used with the lock sample of D_2O contained in a concentric 5 mm tube. The ammonium nitrate reference was contained in a 5 mm tube, with the D_2O lock sample in a concentric 10 mm tube.[20]

pH Measurements were made with a Corning-Eel 7 pH meter. pH Values were adjusted by using HCl and NaOH solutions; the values quoted for the peptides were taken from ref. 21.

All samples used were either commercial or prepared by standard methods. The sample of polyglycine was obtained from Miles Laboratories Inc., and the concentration employed (125 mg in 1 ml of TFA) was equivalent to *ca.* 2M in the glycine residue. All other samples were run as 0.8—1.2M-solutions.

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[20] J. M. Briggs and E. W. Randall, *Mol. Phys.*, 1973, **26**, 699.
[21] 'Data for Biochemical Research,' 2nd edn., eds. R. M. C. Dawson, D. C. Elliott, W. H. Elliott, and K. M. Jones, Clarendon Press, Oxford, 1972.