A Nitrogen-14 Nuclear Magnetic Resonance Study of Amino-acids, Peptides, and Other Biologically Interesting Molecules

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^{14}N Nuclear resonances have been observed in a 7·5 T magnetic resonance spectrometer. 55 Compounds of biological interest were studied in aqueous solutions of concentration *ca.* 200 mM, and the chemical shifts and relaxation times of the ^{14}N resonances recorded. The chemical shifts are discussed in terms of structures, and the relaxation times in terms of nuclear quadrupole coupling constants and molecular correlation times.

BROAD lines, low sensitivity, and low resonance frequency have previously prevented ^{14}N n.m.r. from being applied to the study of biological systems.

However, with the advent of high field superconducting solenoids and accumulation techniques, these difficulties are less formidable. For example, using a 7·5 Tesla superconducting solenoid the ^{14}N resonance of the nitrate ion in a 10 mM aqueous solution of ammonium nitrate has been observed in our laboratory with a signal to noise ratio of 4 : 1, with an accumulating time of 2 h.

A good signal to noise ratio is important for the study of biological systems whose solubilities precluded previous observation. Furthermore, quite apart from limitations of solubility, there are advantages in working at low concentrations because if the viscosity of the solution approaches that of the solvent, then the minimum linewidth is observed,[1,2] and in chemical shift measurements, the correction for the bulk magnetic susceptibility of the sample becomes negligible.

In this investigation the shifts and linewidths of aqueous solutions of amino-acids, peptides, and derivatives have been correlated. From values of the quadrupolar coupling constant provided by n.q.r. or estimated from a comparison with similar molecules, a correlation time, τ_q, is calculated. The large effect on the shift and linewidth of protonation of a nitrogen group is illustrated in the pH titration of histidine and histamine.

EXPERIMENTAL

The measurements were made with a spectrometer[3] using an Oxford Instrument Co. Ltd. superconducting solenoid operating at 7·5 T. The nitrate ion in the aqueous ammonium nitrate standard[4a] resonated at 23·064218 MHz. The spectrometer operated solely in the pulse mode and T_1, T_2 Fourier transform and time-sharing pulse sequences could be produced. Signal averaging was achieved using a Biomac 1000 averager and the data transformed using the analogue Fourier transform device built by D. Hoult.[3,5]

The stability of the applied field was ensured by the use of a deuterium field-frequency lock. The insertion of an external standard, however, served as an additional check. A further check of the measurements, using the time-sharing method, was to record several ' frequency markers ' from the frequency synthesiser to calibrate the sweep and recheck, each time, the position of the reference. The shift and linewidth measurements were obtained by both the time-sharing and the analogue Fourier transform method for each sample. Shift measurements were limited entirely by the linewidths of the resonances.

There has been very little consistency in the choice of a ^{14}N chemical shift reference. The nitrite ion,[6,7] the nitrate ion,[4,8,9] ammonia,[10] and tetra-alkylammonium salts[11] have all been used.

The external secondary standard chosen was 2·0M-trimethyl(phenyl)ammonium chloride; it is found to be a convenient standard with a linewidth of 9 Hz, and consequently less susceptible to saturation than symmetrical alkylammonium salts, when the radio frequency field is optimised for the broader-amino acid lines.

Where solubility permitted, the sample concentrations were 200 mM, dissolved in doubly distilled water, and the pH adjusted to between 7·0 and 7·5. For tyrosine, the nitrotyrosines, cystine, and ethionine the pH was raised to *ca.* 9 to enhance the solubility to a level acceptable for measurement. The pH of penicillamine was adjusted to 4 to prevent decomposition. The amino-acids and peptides

[1] M. Bose, N. Chaterjee, and N. Das, *J. Mol. Spectroscopy*, 1965, **18**, 32.
[2] D. Herbison-Evans and R. E. Richards, *Mol. Phys.*, 1964, **7**, 515.
[3] D. Hoult, D.Phil. Thesis, University of Oxford, 1973.
[4] (*a*) D. Herbison-Evans and R. E. Richards, *Mol. Phys.*, 1964, **8**, 19; (*b*) J. A. Sogn, W. A. Gibbons, and E. W. Randall, *Biochemistry*, 1973, **12**, 2100.
[5] D. Hoult, *J. Magnetic Resonance*, 1973, **9**, 205.
[6] B. E. Holder and M. P. Klein, *J. Chem. Phys.*, 1955, **23**, 1956.
[7] L. O. Anderson, J. Mason, and W. Van Bronswijk, *J. Chem. Soc.* (*A*), 1970, 296.
[8] P. Hampson and A. Mathias, *Mol. Phys.*, 1966, **11**, 541.
[9] M. Witanowski and H. Januszewski, *J. Chem. Soc.* (*B*), 1967, 1063.
[10] R. A. Ogg and J. D. Ray, *J. Chem. Phys.*, 1957, **26**, 1339.
[11] E. D. Becker, *J. Magnetic Resonance*, 1971, **4**, 142.

were chromatographically pure and the measurements were recorded at a sample temperature of 18 °C.

The maximum shift correction for changes of bulk susceptibility between the sample and the reference was calculated to be 0·33 p.p.m. and can therefore be neglected compared with the linewidths encountered.

RESULTS AND DISCUSSION

Chemical Shifts.—Figures 1 and 2 illustrate the spectra obtained. The shifts and other parameters are given in Tables 1—14. It can be seen that the groups directly bonded to the nitrogen atom determine the region in which the shift occurs, but other groups in the molecule, or intermolecular interactions, may cause small perturbations within that region.[4a] For example the extremes of the observed shifts in the α-amino-group in the amino-acids are 332 and 346 p.p.m.

The chemical shifts of amino-acids with more than one nitrogen atom also agree with the observations of Herbison-Evans and Richards.[2] The α-amino-group in lysine (Table 4) has a shift similar to that of leucine as expected, and the shift of the ε-amino-group compares directly with that of ethylamine at 338 p.p.m. measured by Herbison-Evans and Richards.[4a] The small deshielding of the α-amino-group is probably due to the electron-withdrawing effects of the adjacent carboxy-group. Similar shifts have also been observed in ^{15}N resonances.[4b]

All three nitrogen resonances of histidine (Figure 1,

TABLE 1

Aliphatic amino-acids

Compound	Conc. (mM)	pH	pK	Shift (p.p.m.)	Δν (Hz)	T_2 (ms)	(eqQ/h) (MHz)	ε	η (cP)	$10^{11}\tau_q$ (s)
Glycine	204	7·27	2·34 9·60	346	114	2·79	1·25	0·50	1·004	1·43
Alanine	200	7·42	2·35 9·69	332	117	2·74	0·97	0·41	1·006	2·48
Valine	192·3	7·42	2·36 9·62	339	144	2·20	1·10 *	0·45 *	1·06	2·4
L-leucine	205	7·47	2·36 9·60	336	207	1·54	1·10 *	0·45 *	1·09	3·42
Iso-leucine	199	7·47	2·36 9·68	340	213	1·50	1·10 *	0·45 *	1·09	3·51

TABLE 2

Hydroxy-amino-acids

Compound	Conc. (mM)	pH	pK	Shift (p.p.m.)	Δν (Hz)	T_2 (ms)	(eqQ/h) (MHz)	ε	η (cP)	$10^{11}\tau_q$ (s)
Serine	205	7·14	2·21 9·15	340	137	2·32	1·215	0·184	1·008	1·95
Threonine	205	7·05	2·63 10·43	342	137	2·320	1·21 *	0·18 *	1·010	1·95

TABLE 3

Dicarboxylic amino-acids

Compound	Conc. (mM)	pH	pK	Shift (p.p.m.)	Δν (Hz)	T_2 (ms)	(eqQ/h) (MHz)	ε	η (cP)	$10^{11}\tau_o$ (s)
			2·09							
Aspartic acid	204	7·29	3·86 9·82	336	137	2·32	1·28 *,[b]	0·41 *	1·08	1·68
			2·02	339 ($CHNH_2$)	160	1·99	1·28 *,[b]	0·41		1·96
Asparagine	196	7·26	8·8	268 ($CONH_2$)	583	0·545	3·51 *,[j]	0·32 *	1·070	0·93
			2·19							
Glutamic acid	202	7·50	4·25 9·67	333	182	1·75	1·28 *,[b]	0·41 *	1·095	2·23
L-Glutamine	207	7·35	2·17	334 ($CHNH_2$)	172	1·85	1·28 *,[b]	0·41 *	1·088	2·12
			9·13	264 ($CONH_2$)	500	0·64	3·51 *,[j]	0·32 *		0·85

TABLE 4

Amino-acids with basic functions

Compound	Conc. (mM)	pH	pK	Shift (p.p.m.)	Δν (Hz)	T_2 (ms)	(eqQ/h) (MHz)	ε	η (cP)	$10^{11}\tau_q$ (s)
			2·18							
Lysine	202	7·38	8·93	335 (α-NH_2)	225	1·41	1·15	0·35	1·05	3·51
			10·53	342 (ε-NH_2)	192	1·65	0·98	0·64		3·79
			2·13							
Hydroxy-lysine	165	7·20	8·62 9·67	336 (α-NH_2) 348 (ε-NH_2)	340 350	0·936 0·909	1·15 *,[e] 1·11 * (calc.)	0·35 *	1·06	5·24 5·24
			1·82							
L-Histidine	220	7·09	6·0	335 (α-NH_2)	205	1·54	1·27 *,[g]	0·5 *	1·083	2·54
			9·17	197 (imid-N-3)	745	0·427	1·437	0·919		6·03
				147 (imid-N-1)	1000	0·318	3·36	0·134		1·9
			2·17							
Arginine	208	7·45	9·04	335 (α-NH_2)	289	1·1	1·15 *,[e]	0·35 *	1·05	4·30
			12·48	312 (others)	~1000	0·318				

TABLE 5

Aromatic amino-acids

Compound	Conc. (mM)	pH	pK	Shift (p.p.m.)	Δν (Hz)	T_2 (ms)	(eqQ/h) (MHz)	ε	η (cP)	$10^{11}\tau_q$ (s)
			1·83							
DL-Phenyl-alanine	191	7·2	9·14	346	260	1·23	1·351	0·622	1·08	2·65
		9·4		323	1530	0·208	1·351	0·622		15·7
			2·20							
Tyrosine	178	10·56	9·11	312	1870	0·170	1·080	0·407	1·08	32·5
			10·07							
			2·38	343 (α-NH_2)	488	0·65	1·27	0·55	1·10	5·87
Tryptophan	55·5 (max.)	7·15	9·38	293 (>NH)	~1000	0·318	3·01	0·18		2·35

TABLE 6

Sulphur-containing amino-acids

Compound	Conc. (mM)	pH	pK	Shift (p.p.m.)	Δν (Hz)	T_2 (ms)	(eqQ/h) (MHz)	ε	η (cP)	$10^{11}\tau_q$ (s)
			1·14							
L-Cysteine	184	7·32	8·33	335	174	1·83	1·220 [b]	0·475	1·009	2·31
			10·78							
			1·65							
L-Cystine	196	10·58	2·26	333	950	0·335	1·220 [b,h]	0·475 *	1·08	12·60
			7·85							
			9·85							
			1·3 (SO_3H)							
L-Cysteic acid	191	7·20	1·9 (CO_2)	336	222	1·43	1·220 [b,h]	0·475 *	1·009	2·95
			8·70							
L-Methionine	211	7·10	2·28	335	195	1·63	1·15 [b,e]	0·35 *	1·10	3·01
			9·21							
L-Ethionine	164	10·80		341	2200	0·145	1·15 [b,e]	0·35 *	1·09	33·84

TABLE 7

Imino-acids

Compound	Conc. (mM)	pH	pK	Shift (p.p.m.)	Δν (Hz)	T_2 (ms)	(eqQ/h) (MHz)	ε	η (cP)	$10^{11}\tau_q$ (s)
L-Proline	216	7·35	1·99	322	137	2·32	1·623 [c]	0·955	1·00	0·848
			10·60							
L-Hydroxy-proline	210	7·50	1·92	323	216	1·47	1·623 [c,i]	0·955 *	1·02	1·97
			9·73							

TABLE 8

Amino-acid derivatives I

Compound	Conc. (mM)	pH	pK	Shift (p.p.m.)	Δν (Hz)	T_2 (ms)	(eqQ/h) (MHz)	ε	η (cP)	$10^{11}\tau_q$ (s)
			1·94	339 (α-NH_2)	212	1·50	1·15 [b,e]	0·35 *		3·27
DL-Ornithine	220	7·15	8·65						1·10	
			10·76	343 (δ-NH_2)	150	2·12	0·98 *	0·64		3·09
Betaine (*NNN*-trimethylglycine)	208	7·10		328·8	4·0	79·6	0·16 (calc.)		1·01	3·4 (leucine)
DL-α-Amino-butyric acid	211	7·2	2·36	336	141	2·26	9·08 [b,a]	0·41 *	1·01	3·01
			9·6							
			2·15	336 (α-NH_2)	259	1·23	1·15 [b,e]	0·35 *		3·99
DL-αγ-Diamino-butyric acid	209	7·42	8·60						1·03	
			10·53	343 (γ-NH_2)	163	1·95	0·98 *	0·64		3·17
Imidazole	200	7·08	6·95	196	90·1	3·53	3·14	0·135	1·029	1·89
Taurine	205	7·20	1·5	343	82	3·88				
			8·74							

TABLE 9

Amino-acid derivatives II

Compound	Conc. (mM)	pH	pK	Shift (p.p.m.)	Δν (Hz)	T_2 (ms)	(eqQ/h) (MHz)	ε	η (cP)	$10^{11}\tau_q$ (s)
			5·78	345 (α-NH_2)	186	1·71	1·15 [b,e]	0·35 *	1·049	2·87
L-Histamine	200	7·12	9·75	196 (imid-N-3)	399	0·812	1·44 [b,f]	0·92 *		3·13
				144 (imid-N-1)	547	0·492	3·36 *	0·134 *		1·30
3-Nitro-tyrosine	160	10·59		337 (NH_2)	1740	0·183				
				41 (NO_2)	456	0·698				
3,5-Dinitro-tyrosine	156	10·78		~337 (NH_2)	1810	0·176				
Creatine	110	5·8	2·63	~335	1360	0·214			1·06	
			14·3							
Creatinine	190	7·10	4·80	334 (NCH_3, δ-NH<)	800	0·397			1·08	
			9·2	226 (=NH)	920	0·346				
Phospho-creatine	105	7·20	2·78	~310	1515	0·210			1·10	
			4·58							

TABLE 10

Amino-acid derivatives III

Compound	Conc. (mM)	pH	pK	Shift (p.p.m.)	Δν (Hz)	T_2 (ms)	(eqQ/h) (MHz)	ε	η (cP)	$10^{11}\tau_q$ (s)
Urea	200	6·96	0·10	300	221	1·44	3·51	0·323	1·000	0·369
Glucosamine	200	7·21	7·80	327	532	0·598				
Guanidine	200	7·11	13·6	301	341	0·932				

TABLE 11

Peptides

Compound	Conc. (mM)	pH	pK	Shift (p.p.m.)	Δν (Hz)	T_2 (ms)	(eqQ/h) (MHz)	ε	η (cP)	$10^{11}\tau_q$ (s)
Glycylglycine	208	7·06	3·12	350 (NH_2)	210	1·68	1·28	0·41	1·01	2·33
			8·17	262 (NHCO)	1020	0·312	3·03	0·41		2·35
Glycyl-L-tyrosine	135	7·1		348	635	0·501	1·28 *,[b]	0·41 *	1·10	7·79
Glycyl-L-histidine	105	7·2		352 (NH_2)	465	0·684	1·28 *,[b]	0·41 *	1·15	5·71
				187 (imid)	1440	0·221				
Glycyl-methionyl-glycine	118	7·24		348	670	0·497	1·28 *,[b]	0·41 *	1·13	7·85

TABLE 12

Antibiotics and their derivatives

Compound	Conc. (mM)	pH	pK	Shift (p.p.m.)	Δν (Hz)	T_2 (ms)	(eqQ/h) (MHz)	ε	η (cP)	$10^{11}\tau_q$ (s)
			1·8							
Penicillamine	202	3·83	7·9	336	253	1·26	1·22 *,[h]	0·47 *	1·09	3·35
			10·5(SH)							
Benzyl penicillin (Na salt)	150	7·21		~320	2000	0·160	3·03 *,[b]	0·41 *	1·6	4·36
Polymyxin B sulphate	100	7·0		~345	2170	0·147	9·08 * (from αγ-DAB)	0·64 *	1·974	42·5

TABLE 13

Phospholipids and their derivatives

Compound	Conc. (mM)	pH	pK	Shift (p.p.m.)	Δν (Hz)	T_2 (ms)	(eqQ/h) (MHz)	ε	η (cP)
Choline chloride	100	7·1		326·8	5·8	54·5			1·01
Choline phosphate	70	7·0		326·5	5·0	63·0			
Ovolecithin in water-free $CHCl_3$	30			329	38·5	8·26			

TABLE 14

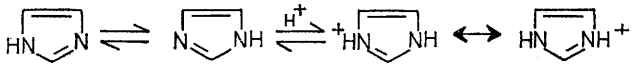
Nucleotides and their derivatives

Compound	Conc. (mM)	pH	pK	Shift (p.p.m.)	Δν (Hz)	T_2 (ms)	(eqQ/h) (MHz)	ε	η (cP)	$10^{11}\tau_q$ (s)
			<1				3·2 (N-7)	0·225		
Adenine	70	3·0	4·1	185	1020	0·312	2·05 (N-9)	0·537	1·038	2·54
			9·8				2·79 (N-10)	0·531		
Adenosine	70	2·2	3·6	~239	2600	0·122	3·15 (N-7)	0·285	1·10	6·28
			12·5				2·95 (N-10)	0·474		
AMP	50	4·2	3·9	~245	5430	0·059	2·95 *,[k]	0·40 *	1·12	13·0
			6·2							
Guanidine	50	Alkali	3·3	182	1220	0·268			1·099	
			9·2							
			12·3							
Guanosine	50	0·5	1·6	195	1700	0·187			1·129	
			9·2							
			12·3							

Footnotes to Tables 1—14

* Denotes assumed eqQ/h and ε. [a] Alanine values used. [b] Glycylglycine values used. [c] Mean value between glycine and alanine. [d] Serine values taken. [e] Lysine values used. [f] Histidine values used. [g] Trytophan values used. [h] Cysteine values used. [i] Proline values used. [j] Urea values used. [k] Adenosine values used.

Table 4) may be observed; the shifts can be compared with those of histamine (Table 9) and imidazole (Table 8).



In imidazole at neutral pH the nitrogen atoms are chemically equivalent because of rapid tautomerism, and resonance hybrids are produced in the protonated form, and one line only is observed. In histidine and histamine the substituent in the '5'-position perturbs the equivalence and two imidazole lines are observed. The shift of the amino-group in histamine is comparable with that of lysine and ornithine.

The secondary amino-group in tryptophan (Table 5)

has a low field shift, probably because of rapid tautomerism with the indolenine derivative, in which the nitrogen has a π-bonded structure. However, the equilibrium

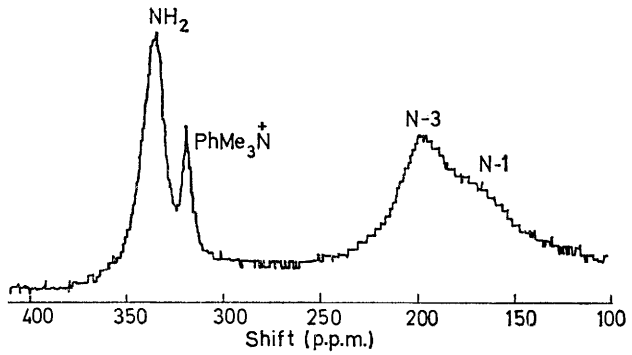


FIGURE 1 ^{14}N N.m.r. spectrum of histidine (200 mM), pH 6·4, 7,500 scans, 1 h

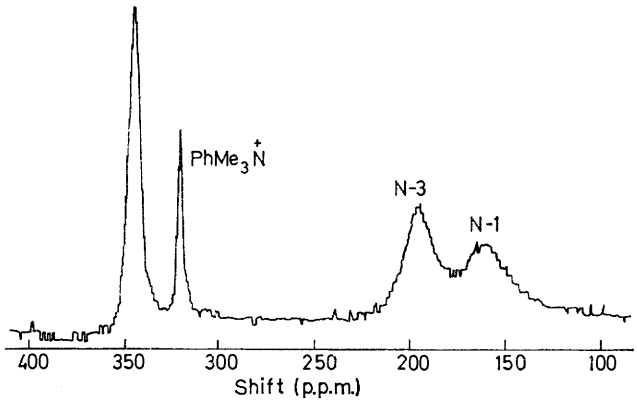
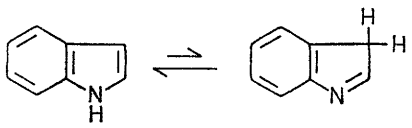


FIGURE 2 ^{14}N N.m.r. spectrum of histamine (200 mM), pH 6·4, 7,500 scans, 1 h

favours the indole structure, and the deshielding contribution from the π-electrons in the indolenine form is,



therefore, small, moving the chemical shift of the indole nitrogen atom in tryptophan to much higher field than in imidazole, but to lower field than in the saturated counterpart, proline (Table 8).

The large linewidth and low concentration of the peptides (Table 11) prevented accurate measurement of the shift, and in all but diglycine the amide nitrogen atom was not observed. The shift of the amide nitrogen in diglycine, downfield from urea, is a result of deshielding from the loss of a bonded hydrogen.

The shifts of the phospholipid, ovolecithin, and its derivatives (Table 13) are comparable with those of the other tetra-alkylammonium shifts measured.[4a]

[12] A. Abragam, 'The Principles of Nuclear Magnetism,' Oxford Univ. Press, Oxford, 1961, p. 314.
[13] W. B. Moniz and H. S. Gutowsky, *J. Chem. Phys.*, 1963, **38**, 1155.
[14] D. T. Edmonds, personal communication.

The antibiotics, penicillin and polymyxin B (Table 12), and the nucleotides (Table 13) produced very broad lines which precluded the measurement of the shifts of the individual nitrogens.

Nitrogen Relaxation Times (Quadrupolar Effects).—In addition to its magnetic moment, the nitrogen nucleus possesses an electric quadrupole moment originating from non-spherically symmetric nuclear charge distribution. This quadrupole moment couples with any electric field gradient at the nucleus to give the so-called nuclear electric quadrupole coupling constant eqQ/h. If the field gradient arises from the electron distribution around the nucleus, it may fluctuate with the motion of the molecule in a liquid, and the resulting modulation of the nuclear quadrupole coupling constant can be a potent mechanism of nuclear relaxation.

In the case of extreme, narrowing, where $\tau_q \ll \omega_0^{-1}$ and ω_0 is the Larmor frequency, for a nucleus with $I = 1$, we write [12] equation (1) where τ_q is the correlation time

$$\frac{1}{T_1} = \frac{1}{T_2} = \frac{3}{8}\left(1 + \frac{\varepsilon^2}{3}\right)\left(\frac{eqQ}{h}\right)^2 \tau_q \qquad (1)$$

representing the reorientations and ε is the electric field asymmetry parameter. In ^{14}N, quadrupolar relaxation usually dominates the nuclear relaxation time, as the magnitude of eqQ is often much larger than the magnetic interaction energies of other relaxation processes.

If the quadrupolar coupling constant is known and T_1 or T_2 have been measured, then a value of τ_q can be derived for the group containing the nucleus. Reliable correlation times for nuclear relaxation in liquids are otherwise difficult to obtain.

Alternatively if a good estimate of τ_q can be obtained, a value for eqQ/h can be derived. Neglecting the asymmetry term ε in the estimation of eqQ/h produces errors which in our case are usually <5%.[13] The values of eqQ/h obtained by microwave methods and by n.q.r. often differ by as much as 10% because intermolecular interactions contribute to the field gradients, and, therefore, the temperature and state of the sample will affect the measured coupling. Edmonds [14] has tried to estimate the intermolecular contribution in the solid by measurement, using n.q.r., of quickly frozen aqueous solutions of amino-acids, utilising progressive dilution.

Estimation of τ_q.—Previous investigators have used viscosity measurements to estimate molecular correlation times, τ_c.[3,13,15-18] Knowing the quadrupolar coupling constants of several of the compounds observed it was then possible to obtain values for a $\tau_c : \tau_q$ ratio. eqQ/h values previously unobserved or too small to be obtained directly could then be estimated from a value of τ_q calculated from the relevant $\tau_c : \tau_q$ ratio. However, the ratio $\tau_c : \tau_q$ varied from 3 to 30 and the values were

[15] R. A. Assink and J. Jonas, *J. Magnetic Resonance*, 1971, **4**, 347.
[16] Dinesh and M. T. Rogers, *J. Magnetic Resonance*, 1972, **7**, 30.
[17] G. J. Jenks, *J. Chem. Phys.*, 1971, **54**, 658.
[18] C. Hall, D.Phil. Thesis, University of Oxford, 1969.

often difficult to systematise, especially for large, polar, non-spherical molecules, as τ_q refers only to reorientations which affect the field gradient.

Provided that the solution viscosity approaches that of water the T_2 observed can be considered equal to the T_2 at infinite dilution but viscosity measurements for all solutions were made nonetheless. At ' infinite ' dilution the correlation times obtained are comparable and an increase in correlation time and so of relaxation rate with molecular size would be expected. The viscosity measurements were performed using an Ostwald viscometer, calibrated with doubly distilled water, at 18·2 °C, the temperature of the sample in the probe. The viscosities measured are tabulated.

The quadrupolar coupling constant and the asymmetry term have been measured for a number of the amino-acids and their derivatives.[19-23] Since eqQ/h is predominantly determined by the atoms bound to the nitrogen atom, it would not be expected to vary much for the α-amino-nitrogen atom, as is the case. We can therefore estimate eqQ/h for the nitrogen atoms in other amino-acids, and the maximum error for the coupling constant at α-nitrogen atoms is estimated as 13%, giving an error in τ_q of 26%. This method has been used to find the correlation times for all the amino-acids and derivatives studied, and is particularly useful for larger molecules, where errors from other methods are very large. The results obtained follow the expected trend; the larger the molecule the longer the correlation time τ_q, (Tables 1 and 2).

Proton exchange at the NH_3 group will effectively modulate the quadrupolar coupling constant, as there will be a difference in eqQ/h values of the protonated and non-protonated forms. The contribution of this effect to the observed relaxation time depends on this difference in eqQ/h, on the exchange rate, and on the fraction of the non-protonated form. We expect that the contribution from exchange is likely to be significant only at pH values near the pK value.

Correlation times for nitrogens in two or more parts of the molecule are obtained for some amino-acids. In Table 4 the τ_q values obtained from the 2- and 5-nitrogen atoms of lysine are very similar as expected, and on the same basis an eqQ/h value for the 5-amino-nitrogen of hydroxylysine can be calculated from the correlation time obtained for the 2-amino nitrogen. The τ_q values obtained for histidine (Table 4) are unusually inconsistent; this might be due to restrictions in the tautomerism of the imidazole ring in the solid at 77 K, at which temperature eqQ/h was measured.

In betaine (Table 8) the narrow linewidth means that quadrupolar relaxation is small and dipolar relaxation may therefore also contribute to T_2. However, assuming it to be small, an approximate value of eqQ/h of 0·16 MHz can be obtained using a τ_q value of $3{\cdot}4 \times 10^{-11}$ s, the value obtained for leucine. Inconsistencies in the τ_q values obtained for tryptophan (Table 5) are in part due to the low solubility and the broad line observed, giving large errors in T_2.

Polymyxin B sulphate (Table 12) is an undecapeptide, six of the residues being 2,4-diaminobutyric acid, and having a total of five possible positive charges. The molecular weight is 1300 and the calculated correlation time of $42{\cdot}5 \times 10^{-11}$ s is comparable with the value of $5{\cdot}0 \times 10^{-10}$ s obtained from ^{1}H n.m.r. for the methyl groups on the leucine residue in polymyxin B.[24,25]

Effect of pH on the Nitrogen Resonances in Aqueous Solutions of Histidine and Histamine.—The three nitrogen resonances in histidine and histamine have been observed (Figures 1 and 2), but the resolution of the imidazole nitrogen atoms is not complete, especially in histidine.

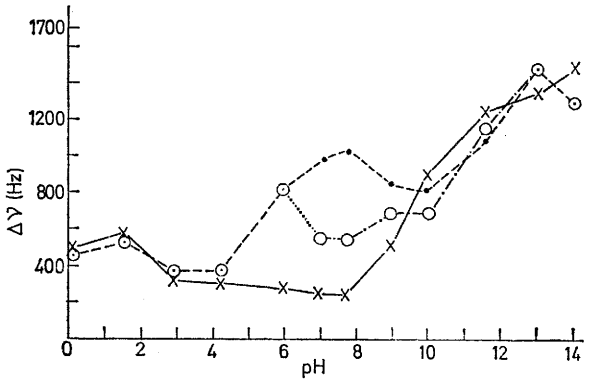


FIGURE 3 Variation of linewidth with pH for histidine (200 mM): ×, α-NH_2 (pK 9·8); ○, imidazole N-3, ●, imidazole N-1 (pK 6·0); ——. CO_2H (pK 1·8)

Assignments of the N-1 and N-3 resonance lines in histidine and histamine are based on the similarity in the shifts of the N-3 nitrogen atoms to that of imidazole; the substituent would be expected to have a greater effect on the N-1 resonance.

The presence of the carboxy-group in histidine causes broadening of all three nitrogen lines, and this is possibly due to a change in the inductive effect of the aliphatic side chain, and a change in the intermolecular hydrogen bonding due to the polar carboxy-group.

Figure 3 demonstrates the effect of the polarity of the carboxy-group (pK = 1·82), on the resonances of both the imidazole nitrogens and the amino-nitrogen. The linewidths can be seen to increase at the acid pK value, and these increases do not occur in the histamine curve (Figure 4) over the same range of pH. Large increases in linewidth are also observed for the nitrogen resonances at their respective pK values, the largest effect being for the α-amino-group, as there is a change from tetrahedral

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symmetry to a distorted structure incorporating the lone pair on deprotonation. The overlap in the imidazole lines of histidine prevented resolution up to pH 6·0, but

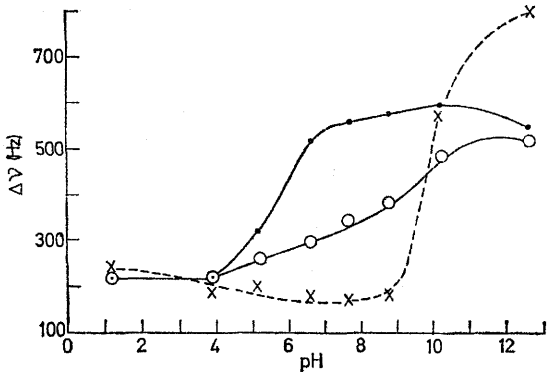

FIGURE 4 Variation of linewidth with pH for histamine (200 mM): ×, α-NH_2 (pK 9·8); ○, imidazole N-3, ●, imidazole N-1 (pK 5·8)

at pH 7—13 the lines were shifted sufficiently to enable partial resolution, which was enhanced by the use of deconvolution of the free induction decay.

Figure 5 illustrates the chemical shift changes in histididine with change in pH. The apparent lack of shift change in the α-amino-nitrogen atom is consistent with the fact that there is a shift change of *ca.* 10 p.p.m. between non-protonated and protonated primary amines.[3] This has also been shown in liquid ammonia compared to the ammonium ion.[3,7] The traverse of the pK value therefore would not yield a significant shift change. The shift change of the imidazole nitrogen atoms follows the expected curve, but the large linewidths obviously preclude observation of small shift changes associated with intermolecular interactions.

Conclusions.—^{14}N Resonances show large chemical shifts and can often be distinguished for chemically different nuclei in molecules of biochemical interest, at sufficiently high applied magnetic field strengths.

However, the nuclear electric quadrupole relaxation of most nitrogen resonances causes broad lines which often overlap and are sometimes too weak to be observed in dilute solution. For relatively small molecules, or others where there is rapid segmental motion, the correlation time is short enough to allow many useful resonances to be observed; polypeptides and proteins will in general have to await still higher magnetic field strengths.

Some nitrogen resonances, particularly from quaternary nitrogen atoms, are characterised by very small quadrupole coupling constants and give very narrow lines. These can be measured even when the correlation

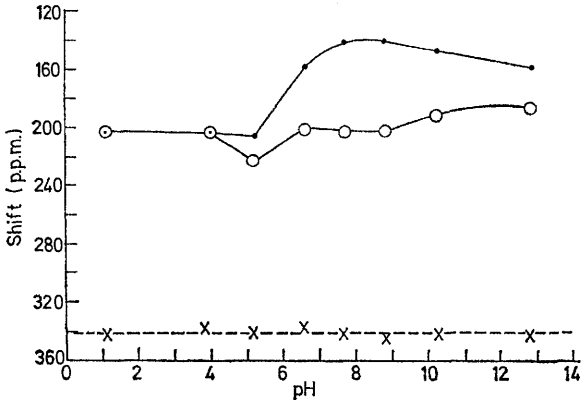

FIGURE 5 Variation of shift with pH for histidine (200 mM): ×, α-NH_2; ●, ○, imidazole

time is long, and may be especially useful in the study of chemical exchange between a small molecule and its complex with a larger one.

Acknowledgement is made to the S.R.C. for a Studentship to N. A. T. We also thank the S.R.C. and the Paul Instrument Fund of the Royal Society for grants in aid of equipment. We gratefully acknowledge helpful discussions with the late Dr. J. D. Bell.

[3/1974 *Received*, 26*th September*, 1973]