Proton Magnetic Resonance Spectra of Amino-acids and Peptides relevant to Wool Structure. Part II.[1] Relative Residence Times of Sulphur-containing α-Amino-acids

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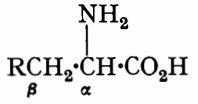
High resolution ^{1}H n.m.r. spectra at 60 MHz of alkaline deuterium oxide solutions of eight sulphur-containing amino-acids have been examined over the temperature range 280—355 K. Iterative analyses of the ABC spin systems of the methylene and methine resonances yield coupling constants from which apparent fractional populations of the individual rotamers have been calculated. For djenkolic acid there is a slight tendency for equalization of population with increase in temperature; for cysteine sulphinic acid and, to a small extent, *S*-carboxymethylcysteine, the dominance of one or two rotamers increases with temperature; for lanthionine, cysteine, and *S*-benzylcysteine, the relative lifetimes of the three rotamers are almost unchanged; while in the case of *S*-methyl- and *S*-allyl-cysteine, the dominant rotamers alter with temperature. For six sulphur-containing amino-acids examined in acidic solution at 303 K, the coupling constants derived are remarkably consistent; the geminal coupling, J_{AB}, is *ca.* −15·0 Hz (modulus about 2 Hz larger than for alkaline solution), and the vicinal couplings, J_{AC} and J_{BC}, are *ca.* 7·9 and 4·4 Hz, respectively.

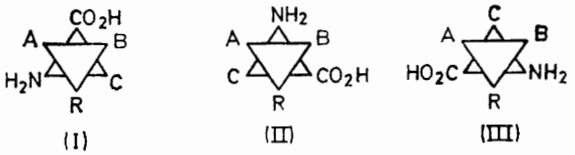
High resolution ^{1}H n.m.r. spectroscopy provides, in principle, a sensitive magnetic probe of the chemical nature, magnetic environment, and motion of the hydrogen atoms in a biological macromolecule and hence of its conformation in solution. ^{1}H N.m.r. spectra of amino-acids and dipeptides[1,2] are valuable, not only intrinsically for their information about rotational isomerism, but also as a basis towards interpretation of overlapping spectra from proteins such as wool.[3,4] In addition to the variation of chemical shifts of amino-acid and other protons with ionization state (zwitterion forms are usual in neutral solution), more detailed ^{1}H n.m.r. spectroscopic analyses of the methylene (β) and methine (α) protons of α-amino-acids (A) can reveal



(A)

variations in the vicinal coupling constants. Protons in the R group are presumed not to spin–spin couple to the α- and β-protons. Earlier studies of the spectra of sulphur-containing amino-acids[1] in acid solution at 303 K have been extended to alkaline solutions and temperatures from 283 to 353 K. From spectral analyses, fractional populations of rotational isomers have been deduced in these and other amino-acids.

Rotational Isomerism about the C_α–C_β *Bond.*—In solution, an α-amino-acid may be expected to exist as an equilibrium mixture of three rapidly interconverting rotamers, staggered about the C_α–C_β bond, represented by the Newman projections (I)—(III) down C_β–C_α. Even if β-hydrogens A and B spend equal times in the three conformations, chirality of the α-carbon atom ensures that A and B are not magnetically equivalent.[5] Accordingly, in 1,1,2-trisubstituted ethanes, the aliphatic



(I) (II) (III)

spectrum of each rotamer should conform to an ABX/ABC spin system, with up to fourteen lines, from which two vicinal coupling constants can be extracted; fast rotation should yield a single ABC spectrum for the set of isomers.[5] If hydrogens A and B have fortuitously identical shifts, then the spin system[6] becomes AA'B or AA'X, with coupling constants J_{AB}, $J_{A'B'}$, and $J_{AA'}$.[7] Since, in acidic solution, certain amino-acids give a simple five-line aliphatic 60 MHz spectrum which may retain this appearance for trifluoroacetic acid (TFA)

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solutions at 220 MHz,[8] intrinsic differences between A and B protons can be very small. Where a system is deceptively simple[9] with an X triplet and an AB doublet (*i.e.* of similar appearance to an A_2X in which there is only one vicinal coupling, J_{vic}), individual couplings cannot be extracted, since the line separation is $\frac{1}{2}|J_{AX} + J_{BX}|$.

Electronegativity corrections[2] to Karplus'[10] notion of J_{vic} as a function of the C–H dihedral angle are important for 1,2-dihalogenoethanes. The gauche coupling in the *trans*-isomer can differ[11] from the (two different) gauche couplings in the gauche-isomer; similarly, *trans*-couplings in the *trans*- and gauche-isomers can differ. With the assumption[12,13] of one gauche (J_g) and one *trans* (J_t) coupling (and also, strictly,[2] that each J_{vic} extracted can be associated with the individual methylene proton), lifetimes for the conformers can be deduced. This simplification need not affect calculated lifetimes greatly, nor prevent one from following *changes* in residence times, provided, of course, that two J_{vic} values can be extracted. It yields Pachler's[12] equations for the relative residence times *a*, *b*, and *c* of the conformers (I), (II), and (III) in terms of experimental values J_{AC} and J_{BC} for J_{vic}; J_t and J_g are taken to be 13·6 and 2·6 Hz, respectively.[13,14]

EXPERIMENTAL

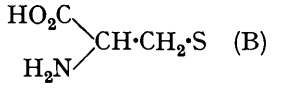
Materials.—*S*-Carboxymethylcysteine was purchased from Mann Research Laboratories and L-cysteine from Sigma London Chemical Co. Ltd. Samples of cysteine sulphinic acid, *S*-allylcysteine, *S*-benzylcysteine, *S*-methylcysteine, L-djenkolic acid, and DL-lanthionine were purchased from Nutritional Biochemicals Corp. The solvents were deuterium oxide (99·7% isotopic purity) from Prochem Ltd., sodium deuterioxide (40% solution in 99% deuterium oxide) from Fluka AG, and deuteriated trifluoracetic acid (99% deuteriated) from CIBA (ARL) Ltd. Sodium 3-trimethylsilylpropane-1-sulphonate (Merck) was used as internal reference. Acidities, pD, were obtained[15] by adding 0·4 to the pH measurement made on an EIL GHM 23/B meter with a glass electrode and potassium bromide salt bridge.

Spectra.—In general, spectra were recorded for 0·5M-solutions of amino-acids in deuterium oxide, typically 1·0M in sodium deuterioxide, although some measurements were made in 10% w/w $CF_3{\cdot}CO_2D$. All were in 5 mm sample tubes on a Varian A-60 spectrometer equipped with a Varian V-6057 variable-temperature probe. Before and after measurements, the probe temperature was measured from the line separation in the spectrum of ethylene glycol and the Varian graph of chemical shift *vs.* temperature. Samples were equilibrated at probe temperature for 10 min. Spectrometer conditions were: sweep rate, 500 Hz s^{-1}; sweep widths, 500 or 100 Hz; filter bandwidths 1·0 or 0·4 Hz; and r.f. level 0·03—0·04 mG. Three to six replicate spectra were recorded at each temperature.

Calculations.—Apart from some deceptively simple[9] ABC systems, all the spectra were analysed as ABC systems to give the parameters in Tables 1 and 2; J_{AC} and J_{BC} are of opposite sign to J_{AB}, which is assumed to be negative. Trial ν and *J* values from an ABX approximation allowed line assignment for analysis by the iterative program LAME, written in Fortran IV by Mr. C. W. Haigh, for six spins for an I.C.T. 1905 computer and modified for four spins for the Bradford University I.C.T. 1909 computer; this program requires less storage than LAOCOON.[16] In a well resolved line of half-height width $<0{\cdot}8$ Hz, a 0·1 Hz error corresponds to errors of 0·1 Hz in ν and rather less in *J*; ν_A and ν_B, recorded on the same 100 Hz sweep, should retain this relative accuracy; ν_C, sometimes recorded on separate sweeps, is not strongly correlated with the other parameters. While negative correlations between ν_A and ν_B were occasionally as high as $|0{\cdot}7|$, standard deviations remained in the same range, 0·02—0·10 Hz, as for the other parameters, between which correlations were usually $<|0{\cdot}2|$. In particular, except occasionally between themselves, J_{BC} and J_{AC} were only slightly correlated with other parameters. For some sulphur-containing acids, with weak lines in the AB region, the J_{AB} error may be as high as 0·2 Hz.

RESULTS AND DISCUSSION

Side-chain Rotation in Sulphur-containing Amino-acids.—For sulphur-containing amino-acids containing the group (B) Table 1 lists spectral parameters, together



(B)

with fractional rotamer populations deduced from them by the Pachler procedure.[13] Since the electronegativities of sulphur and carbon are closely similar, inclusion of S should have little influence on the net electronegativity; thus values estimated for the average *trans*–gauche coupling, J_{av}, for other amino-acids should be valid here.[13,14] Previous analyses[17] of the spectra of compound (3), generally by the less exact ABX method, and with coupling-constant arguments somewhat analogous to those of Pachler, favour the predominance of rotamer (I) in alkaline (pH 10·3 and 12·7;[18] pH > 11[19]) and acid (AB_2 analysis; pH < 0)[19] solution. From chemical shifts, Martin and Mathur[19] emphasised the influence of charge [SH ionization is favoured in (3) and ammonium ionization in (5)] but, with AB_2 spectra at all pH values, were inconclusive about rotamer populations in (5).

Cysteine (3) (183·5 Hz) and cysteine sulphinic acid (4) (210·5 Hz) apart, the methine shift, ν_C, at ambient temperature, is within the range 200—207 Hz. Similarly, apart from djenkolic acid (2) (175·7 Hz) and the

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TABLE 1

Temperature dependence of ^1H n.m.r. spectral parameters and rotamer populations for sulphur-containing amino-acids in alkaline solution [a]

	Chemical shifts (Hz) [b]				J/Hz			Fractional rotamer populations			
T/K	ν_A	ν_B	ν_C	$\nu_B - \nu_A$	J_{AB}	J_{AC}	J_{BC}	a	b	c	Other chemical shifts (Hz) [b]
					(1) Lanthionine (Cy·S·Cy) [c] (pD 12·9)						
283	164·0	167·9	202·0	3·9	−13·1	7·0	5·0	0·40	0·22	0·38	
303	164·5	170·4	201·6	5·9	−13·0	6·8	5·05	0·38	0·23	0·39	
333	164·4	172·1	201·2	7·7	−13·0	7·15	5·0	0·41	0·22	0·37	
353	163·9	172·7	201·2	8·8	−13·0	7·25	4·95	0·42	0·21	0·37	
					(2) Djenkolic acid Cy·S·CH_2·S·Cy (pD 13·2)						
											S·CH_2·S
283	(ν_{AB})177·0		210·0			5·9					226·0
303	173·4	178·0	205·0	4·6	−13·0	6·9	4·5	0·39	0·17	0·44	
333	170·3	177·3	204·3	7·0	−13·2	7·0	5·0	0·40	0·22	0·38	226
353	169·9	178·8	203·8	8·9	−13·1	7·15	5·0	0·41	0·22	0·37	226
					(3) Cysteine Cy·SH (pD 13·2)						
283	148·4	171·2	183·5	22·8	−12·2	8·8	3·2	0·56	0·07	0·37	
303	148·2	171·0	183·3	22·8	−12·1	8·8	3·2	0·56	0·07	0·37	
333	148·2	170·9	183·3	22·7	−12·1	8·8	3·2	0·56	0·07	0·37	
353	148·0	170·8	183·2	22·8	−12·2	8·8	3·2	0·56	0·07	0·37	
					(4) Cysteine sulphinic acid Cy·SO_2H (pD 11·9)						
283	152·0(ν_{AB})		212·0			6·7		0·33	0·33	0·33	
303	149·5	153·4	210·5	3·9	−13·0	7·7	5·9	0·42	0·26	0·32	
333	149·8	154·6	211·1	4·8	−12·9	8·4	4·5	0·48	0·14	0·38	
353	149·5	154·9	211·5	5·4	−13·0	8·6	3·5	0·49	0·07	0·44	
					(5) *S*-Methylcysteine Cy·SMe (pD 13·0)						
											SCH_3
283	161·5	166·4	202·5	4·9	−13·6	6·5	4·9	0·35	0·21	0·44	125
303	161·7	167·7	202·8	6·0	−13·6	7·2	4·9	0·42	0·21	0·39	125
333	161·9	169·4	203·4	7·5	−13·6	7·2	4·8	0·42	0·20	0·38	125
353	162·6	170·4	203·2	7·8	−13·6	7·4	5·0	0·44	0·22	0·34	125
					(6) *S*-Benzylcysteine Cy·SCH_2Ph (pD 12·8)						
303	162·7	172·6	205·9	9·9	−13·3	7·6	4·9	0·44	0·20	0·36	
333	161·7	172·1	203·1	10·4	−13·0	7·3	4·8	0·42	0·18	0·40	
353	161·1	172·1	202·0	11·0	−13·1	7·4	4·9	0·43	0·20	0·37	
					(7) *S*-Allylcysteine Cy·SCH_2·CH:CH_2 (pD 12·9)						
283	163·3	167·5	206·3	4·2	−13·0	6·0	6·0	0·31	0·31	0·38	
303	159·8	166·4	200·5	6·6	−13·2	6·8	5·1	0·39	0·23	0·38	
333	160·4	168·2	201·5	7·8	−13·2	7·1	5·1	0·41	0·23	0·36	
353	159·4	169·1	199·9	9·7	−13·2	7·3	5·0	0·42	0·23	0·37	
					(8) *S*-Carboxymethylcysteine Cy·S·CH_2·CO_2H (pD 10·9)						
											CH_2·CO_2H
283	168·1	175·2	210·3	7·1	−13·3	7·3	5·1	0·41	0·21	0·38	195
303	166·8	174·9	207·0	8·1	−13·3	7·5	4·9	0·42	0·20	0·38	193
333	165·1	174·1	205·9	9·0	−13·3	7·6	4·7	0·43	0·20	0·39	193
353	164·0	173·9	204·3	9·9	−13·2	7·7	4·5	0·45	0·16	0·39	192

[a] Solutions in D_2O are 0·5M in amino-acid, 1·0M in NaOD. [b] Downfield from sodium 3-trimethylsilylpropane-1-sulphonate (internal reference). [c] Cy = CH_2·$CH(NH_2)$·CO_2H.

TABLE 2

^1H N.m.r. spectral parameters for sulphur-containing amino-acids in acid solution [a] at 303 K

	Chemical shifts (Hz) [b]					J/Hz		
	ν_A	ν_B	ν_C	$\nu_C - \nu_{AB}$	ν_{AB}	J_{AB}	J_{AC}	J_{BC}
(4) Cy·SO_2H	189·9	198·6	277·3	83·1	194·2	−14·8	8·2	4·2
(5) Cy·SMe	186·6	191·7	258·7	69·6	189·1	−15·0	7·6	4·6
(6) Cy·SCH_2Ph	184·7	190·4	257·1	69·5	187·2	−15·1	7·9	4·4
(7) Cy·S·CH_2·CH:CH_2	184·3	191·4	258·6	70·6	187·9	−15·0	7·9	4·4
(8) Cy·S·CH_2CO_2H	191·5	199·4	259·9	64·5	195·4	−15·0	7·9	4·4
(9) Cy·S·$CONH_2$ [c]	205·9	215·4	264·6	54·0	210·6	−15·6	6·6	4·2

[a] Solutions are 10% (w/w) amino-acid in D_2O containing 10% (w/w) CF_3·CO_2D. [b] Shifts of A, B, C are in Hz downfield from sodium 3-trimethylsilylpropane-1-sulphonate (internal reference). [c] *S*-Carbamoylcysteine.

sulphinic acid (4) (151·5 Hz), the mean methylene shifts, ($\nu_A + \nu_B$)/2, of all the acids in Table 1 fall within the range 160—171 Hz at room temperature. The 15 Hz upfield shift in the average frequency of the methylene protons in the acid (4) to *ca.* 152 Hz, close to the 149 Hz of methylene protons in aspartic acid under similar

conditions, is attributed to ionization of the SO_2H group; in *S*-carboxymethylcysteine (8) with its second ionizable group, $S{\cdot}CH_2{\cdot}CO_2H$, the $S{\cdot}CH_2$ group separates the

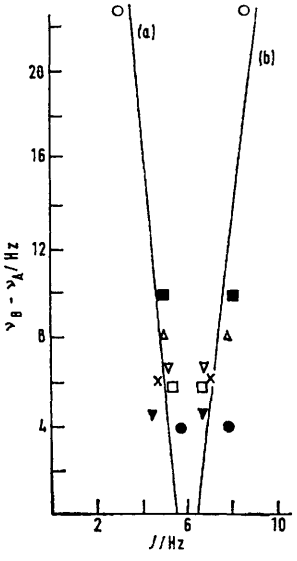

FIGURE 1 Chemical-shift difference, $\nu_B - \nu_A$, *vs.* coupling constants, J_{BC} (a) and J_{AC} (b), for sulphur-containing amino-acids in 1·0M-NaOD solution. □, Lanthionine; ▼, djenkolic acid; ○, cysteine; ●, cysteinesulphinic acid; ×, *S*-methylcysteine; ■, *S*-benzylcysteine; ▽, *S*-allylcysteine; and △, *S*-carboxymethylcysteine

carbonyl from the β-methylene group. When $(\nu_B - \nu_A)$ is plotted against J_{BC} and J_{AC} (room-temperature data) the points for compound (4) are most divergent from the (non-intersecting) least-squares lines (Figure 1), presumably owing to electronegativity differences from the SO_2H group; 6·1 Hz is close to Pachler's value [13] of 6·25 Hz for the average coupling constant, J_{av}, of the $C{\cdot}CH_2{\cdot}CH(NH_2){\cdot}CO_2^-$ fragment. At 303 K, the chemical-shift difference $(\nu_B - \nu_A)$ between the methylene protons increases from 3·9 for compound (4), through 6·0 for compound (5), with its compact methyl group, and values in the range 6—8 for most of the acids, to 9·9 for the *S*-benzyl compound (6) and 22·8 Hz for cysteine (3). In addition to possessing a bulky group tending to hinder rotation, compound (6) may exhibit different ring-current effects on the methylene protons in the three rotamers.

Except for compound (3), corresponding fractional rotamer populations for these amino-acids are remarkably consistent at room temperature, but the temperature dependences display considerable variation (Table 1) Invariance of the spectrum of compound (3) with temperature could be a consequence of ionization of the SH group to S^-; the high population of rotamer (I) and the low population of rotamer (II) may then be attributed to electrostatic repulsion between the S⁻ and CO_2^- groups in (II). This is also likely to be why $(\nu_B - \nu_A)$ is large in compound (3). Increase of temperature to 353 K may not increase the energy enough to alter the relative stability of the rotamer system. Similar behaviour was observed for aspartic acid [18] and for cysteic acid at several pD values; [20] two negative charges may stabilize rotamer (I). In lanthionine (1) also, the rotamer population is almost independent of temperature. Compounds (5), (7), and (8), all experience an increase in population of rotamer (I); this is at the expense of rotamer (III) for (5), and of (II) for (7) and (8). For compound (4), increase in both *a* and *c* occurs at the expense of *b*. For *S*-carboxymethylcysteine (8), convergence of the lines (Figure 2) of J_{AC}, J_{BC} *vs.* $(\nu_B - \nu_A)$ to $J_{av} = 6{\cdot}6_5$ Hz is used to evaluate the rotamer population; phenylalanine behaves in much the same way under similar conditions.[21,22] In both compounds (1) and (6), progressive increase in $(\nu_B - \nu_A)$ with temperature is not matched by systematic changes in coupling constants; for compound (2), this effect is so pronounced that the 60 MHz spectrum has the appearance of an A_2B system at 283 K.

Earlier [1] ambient temperature measurements made in acidic solution for compounds (1)—(3) and other sulphur-containing amino-acids have been extended to acids (4)—(8) and to *S*-carbamoylcysteine (9) (Table 2). Except possibly for lanthionine (1) and *S*-carbamoyl cysteine (9), the coupling constants form a remarkably coherent group. As expected, the geminal coupling,

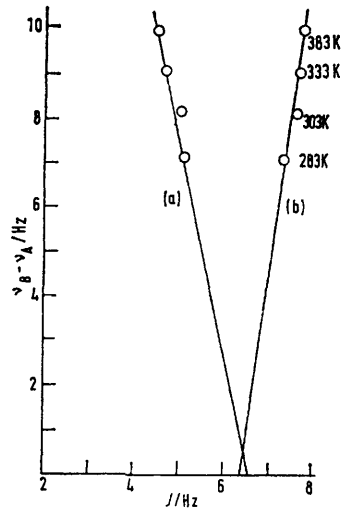

FIGURE 2 $\nu_B - \nu_A$ *vs.* J_{BC} (a) and J_{AC} (b), for *S*-carboxymethylcysteine in 1·0M-NaOD solution

J_{AB} 15·0 Hz, has a modulus *ca.* 2 Hz larger than in alkaline solution. The greater divergence between the vicinal coupling constants J_{AC} *ca.* 7·9 and J_{BC} *ca.* 4·4 Hz than in alkaline solution corresponds to more disparate populations.

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