

**723.** *The Proton Resonance Spectra and Conformations of the Prolines. Part III.<sup>1</sup> Hydroxyproline and Allohydroxyproline in Acid and Alkaline Solution.*

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The proton resonance spectra of hydroxyproline and allohydroxyproline in strongly acid and alkaline solutions are reported and analysed. The conformations of the molecular ions in these solutions are deduced from the observed coupling constants. A number of possible internal reference compounds for such media have been measured against the methyl-group shift of sodium 2,2-dimethyl-2-silapentane-5-sulphonate and shown to give a consistent chemical-shift scale.

THE determination of the conformations of cyclic compounds in solution from their proton resonance spectra is now a well-recognised technique. It is based on the use of the equation, originally proposed by Karplus,<sup>2</sup> which relates the proton coupling constants in a CH-CH fragment to the dihedral angle,  $\phi$ , between the relevant C-H bonds (eqn. 1), which in turn depends on the molecular conformation.

$$J = \begin{matrix} k_1 \cos^2\phi - C & 0^\circ \leq \phi \leq 90^\circ \\ k_2 \cos^2\phi - C & 90^\circ \leq \phi \leq 180^\circ \end{matrix} \quad (1)$$

These vicinal coupling constants are also dependent on the electronegativity of the substituents attached to the C-C fragment;<sup>3-5</sup> thus it is necessary to determine empirically the values of  $k_1$ ,  $k_2$ , and  $C$  for each fragment, as well as  $\phi$ . These were obtained in Parts I<sup>6</sup> and II<sup>1</sup> of this Series for the two isomeric 4-hydroxyprolines in neutral solution, and, from the values of  $\phi$ , the conformations of the molecules in solution were deduced.

<sup>1</sup> Part II, Abraham and McLauchlan, *Mol. Phys.*, 1962, **5**, 513.

<sup>2</sup> Karplus, *J. Chem. Phys.*, 1959, **30**, 11.

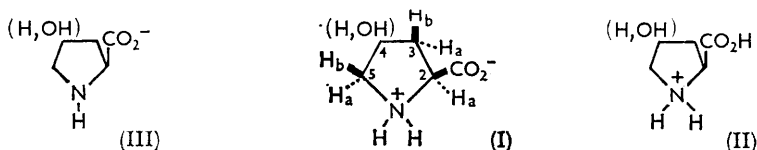
<sup>3</sup> Glick and Bothner-By, *J. Chem. Phys.*, 1956, **25**, 362.

<sup>4</sup> Banwell and Sheppard, *Discuss. Faraday Soc.*, 1962, **34**, 115.

<sup>5</sup> Abraham and Pachler, *Mol. Phys.*, 1963, **7**, 165.

<sup>6</sup> Abraham and McLauchlan, *Mol. Phys.*, 1962, **5**, 195.

In this Paper, these investigations have been extended to acid and alkaline solutions of these compounds. In strongly acid ( $\text{pH} < 0.5$ ) or strongly alkaline ( $\text{pH} > 11.5$ ) solutions, these amino-acids will exist almost entirely as the cation (II) and anion (III), respectively ( $\text{p}K_1 = 1.92$ ,  $\text{p}K_2 = 9.73$  for hydroxyproline,<sup>7</sup> and  $\text{p}K_1 = 1.98$ ,  $\text{p}K_2 = 9.60$  for allo-hydroxyproline<sup>8</sup>).



No other ionic species is of any significance in aqueous acid, as the ionisation constant of the acid  $\text{ROH}_2^+$  is much too high. This is consistent with the experimental results. However, in strongly alkaline solution there is the possibility of the dissociation of the hydroxyl group to give  $\text{RO}^-$ . Assuming the basic strength of this hydroxyl group to be comparable with that in ethylene glycol ( $\text{p}K 14.2$ ),<sup>7</sup> then the  $\text{pH}$  of the alkaline solution must be controlled between 11.5 and 13.0 in order that the proton resonance spectrum of the ion (III) may be obtained.

It is of interest to compare the proton chemical shifts of the three ionic species (I), (II), and (III). To do this, the shifts must all be measured from those of an internal reference. (The use of an external reference necessarily involves a correction for the bulk susceptibility of these concentrated ionic solutions, a procedure which is too inaccurate for the present investigation.) Many internal reference compounds have been suggested for use in aqueous solutions, *e.g.*, *t*-butyl alcohol,<sup>9</sup> tetramethylammonium salts,<sup>10</sup> and acetonitrile.<sup>9</sup> However, in hardly any of these investigations was the effect of strong acid or alkali on these compounds considered. Tiers, in one of the few detailed studies,<sup>11</sup> suggested the use of sodium 2,2-dimethyl-2-silapentane-5-sulphonate (DSS) and proposed a new scale of chemical shifts for aqueous solutions ( $\tau'$ ) based on the value 10.000 for the methyl-group shift in this compound. The reliability of this and of the other suggested reference compounds has been tested by determining their  $\tau'$  values in strongly acid and alkaline solutions.

#### EXPERIMENTAL

The hydroxy-L-proline was obtained commercially and the allohydroxy-D-proline was obtained from Dr. A. V. Robertson.\* The solutions in 3*N*-NaOD and 3*N*-DCl were made as concentrated as possible (*ca.* 300 mg./ml.), in order to observe all the weak lines in the spectrum. The  $\text{pH}$  of the acid solutions was always less than 1 and that of the alkaline solutions 12.9. The spectra were recorded on a Varian A-60 spectrometer, with sample temperatures of *ca.* 30° and DSS as the internal reference. The sweep-widths were continually checked by means of side-band calibration. The experimental errors are estimated to be  $\pm 0.1$  c./sec. for the coupling constants, and  $\pm 0.01$  p.p.m. for the chemical shifts. The high-temperature spectra were obtained in a similar manner, using the Varian V-6057 variable-temperature system. The accuracy of the temperature controller is quoted as  $\pm 2^\circ$ . Several possible reference compounds were measured in the same dilute solution (*ca.* 2% v/v) in  $\text{D}_2\text{O}$ , 3*N*-acid, and 3*N*-alkali; these all gave single sharp peaks, except the tetramethylammonium ion which gave a 1 : 1 : 1 triplet with  $J(\text{CH-N}) 0.60$  c./sec. They were all measured by means of the side-band calibration method, the experimental error of the  $\tau'$  values being less than  $\pm 0.005$  p.p.m.

\* To avoid confusion, the figures for the allohydroxyproline are for the L-stereoisomer, not for the experimentally measured D-form. Of course, the spectra of these are identical.

<sup>7</sup> Hodgman, "Handbook of Chemistry and Physics," Chemical Rubber Publ. Co., Cleveland, 1962.

<sup>8</sup> Greenstein and Winitz, "Chemistry of the Amino Acids," Wiley, London, 1961, Vol. I.

<sup>9</sup> Jones, Katritzky, Murrell, and Sheppard, *J.*, 1962, 2576.

<sup>10</sup> Grunwald, Loewenstein, and Meiboom, *J. Chem. Phys.*, 1957, 27, 641.

<sup>11</sup> Tiers, personal communication.

## RESULTS

The proton resonance spectra of hydroxyproline in acid and alkaline solution are shown in Figs. 1 and 2, respectively, and the corresponding spectra of the allo-isomer in Figs. 3 and 4. The hydroxyl, carboxyl, and amine protons exchange rapidly with the solvent, so that the corresponding peaks coalesce with the residual solvent peak. Thus the complex spectra shown are due to the remaining six protons. The assignment of the main groups in the spectra is straightforward, and is given in Tables 1 and 2. Each spectrum consists

TABLE 1.  
Proton chemical shifts and coupling constants for hydroxyproline in acid, neutral,  
and alkaline solution.

Coupling	Coupling constants (c./sec.)		Proton	Chemical shifts ( $\tau$ )		
	Acid and neutral	Alkaline		Acid	Neutral	Alkaline
$J_{2a-3a}$ .....	7.66	7.1	$H_{2a}$ .....	5.29	5.66	6.31
$J_{2a-3b}$ .....	10.44	9.4	$H_{3a}$ .....	7.48	7.60	7.94
$J_{3a-4b}$ .....	1.41	2.4	$H_{3b}$ .....	7.66	7.88	8.12
$J_{3b-4b}$ .....	4.31	6.1	$H_{4b}$ .....	5.26	5.35	5.58
$J_{4b-5a}$ .....	1.22	3.0	$H_{5a}$ .....	6.55	6.66	7.31
$J_{4b-5b}$ .....	4.09	5.3	$H_{5b}$ .....	6.37	6.52	6.76
$J_{3a-3b}$ .....	14.06	13.6				
$J_{5a-5b}$ .....	12.69	11.9				
$J_{3a-5a}$ .....	1.6	0.8				

of an ABXR system, made up of  $H_{3a}$ ,  $H_{3b}$ ,  $H_2$ , and  $H_4$ , and an ABX system, made up of  $H_{5a}$ ,  $H_{5b}$ , and  $H_4$ . The detailed analysis of these systems is well known and has been given in Part I.<sup>6</sup> From this, the chemical shifts of, and coupling constants between, all these protons are obtained. The only exceptions are the  $H_5$  protons of allohydroxyproline in acid (Fig. 3). In this case, the chemical shift between the  $H_5$  protons is so small that we have an example of a deceptively simple spectrum<sup>12</sup> which does not give a unique analysis. The spectra calculated from the molecular parameters are shown underneath the observed spectra, and the agreement between the two provides a rigorous proof of the analysis. The proton coupling constants for the hydroxyprolines in acid solution were found to be the

TABLE 2.  
Proton chemical shifts and coupling constants for allohydroxyproline in acid, neutral,  
and alkaline solution.

Coupling	Coupling constants (c./sec.)		Proton	Chemical shifts ( $\tau$ )		
	Acid and neutral	Alkaline		Acid	Neutral	Alkaline
$J_{2a-3a}$ .....	10.48	9.5	$H_{2a}$ .....	5.31	5.79	6.52
$J_{2a-3b}$ .....	3.48	6.1	$H_{3a}$ .....	7.43	7.52	7.65
$J_{3a-4a}$ .....	4.71	5.9	$H_{3b}$ .....	7.57	7.78	8.30
$J_{3b-4a}$ .....	2.09	3.3	$H_{4a}$ .....	5.32	5.43	5.67
$J_{4a-5a}$ .....	0.94	2.9	$H_{5a}$ .....		6.58	7.08
$J_{4a-5b}$ .....	4.57	4.8	$H_{5b}$ .....	6.48	6.64	7.23
$J_{3a-3b}$ .....	14.23	13.6				
$J_{5a-5b}$ .....	12.50	12.1				
$J_{3b-5a}$ .....	2.0	1.2				

same, within experimental error, as those previously obtained for the neutral solutions. As the latter spectra are somewhat better resolved, the spectra for the acid solution were calculated by using these more accurate coupling constants.

Although the analysis of the spectra is complete, the detailed assignment is not, as there are two possible assignments for the peaks due to the  $H_{3a}$  and  $H_{3b}$  protons, and for those due to the  $H_{5a}$  and  $H_{5b}$  protons. The assignments of these protons for the neutral solutions have been determined from a consideration of the conformations of the molecules.<sup>1</sup> The coupling constants for the acid and neutral solutions being identical, it follows that

<sup>12</sup> Abraham and Bernstein, *Canad. J. Chem.*, 1961, **39**, 216.

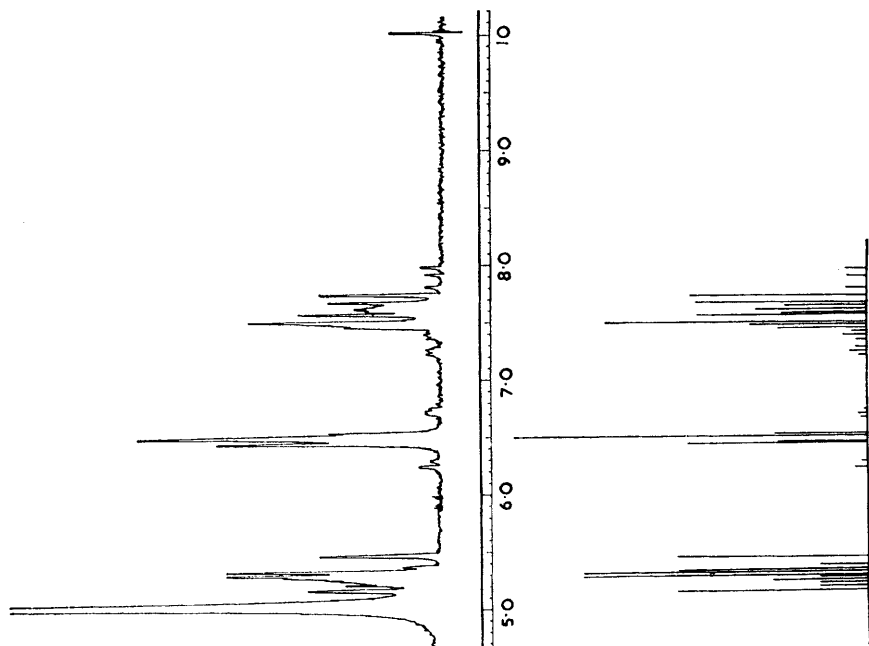


FIG. 1. Proton resonance spectra of hydroxyproline. In 3N-DCl solution, with DSS as the internal reference.

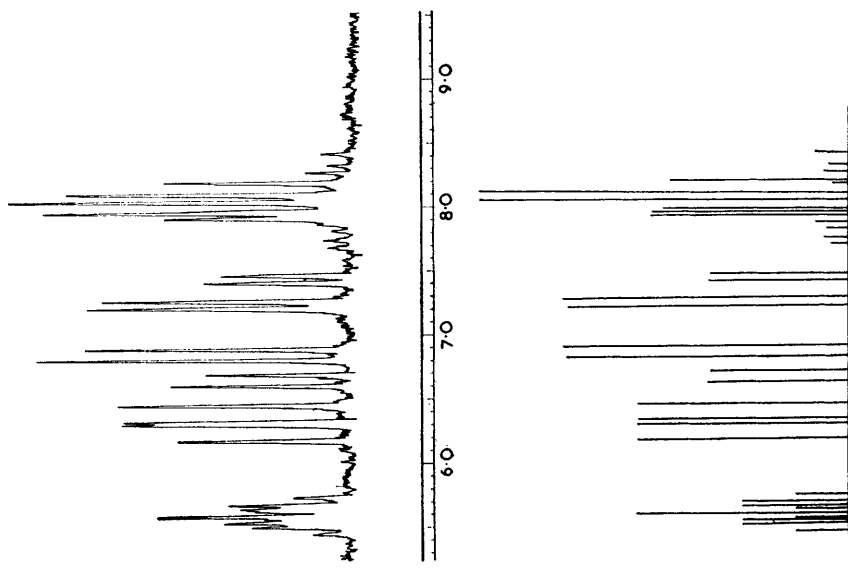


FIG. 2. In 3N-NaOD solution, measured from the DSS internal reference (not shown).

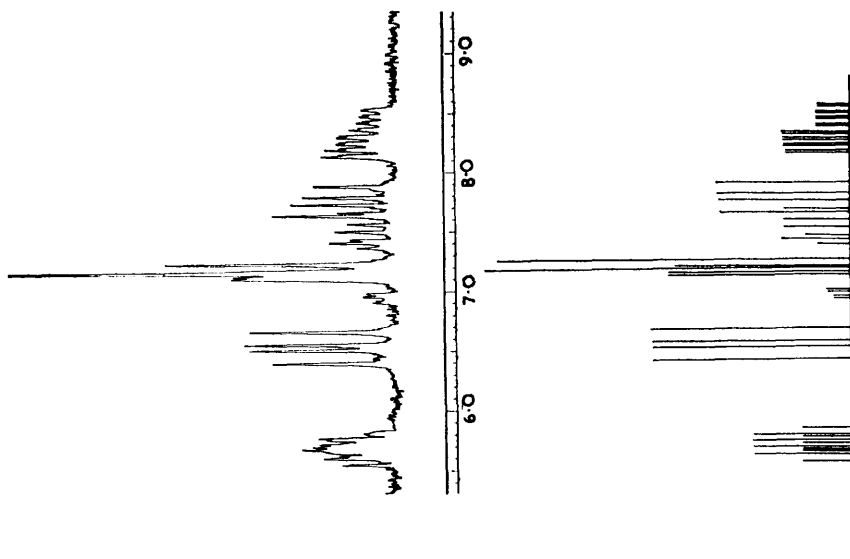


Fig. 4. In 3N-NaOD solution.

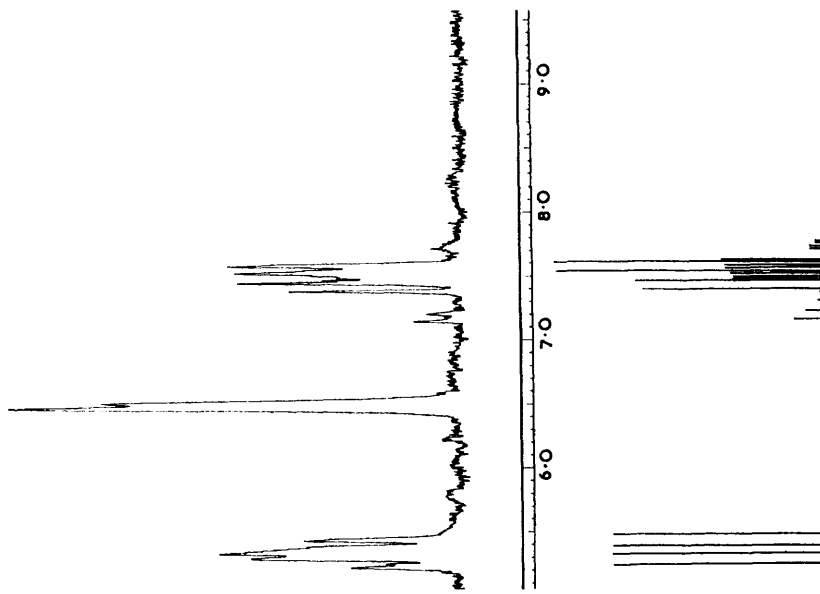


Fig. 3. Proton resonance spectra of allohydroxyproline. In 3N-DCI solution.

the assignments are also the same. However, the coupling constants of the compounds in alkaline solution differ considerably from those observed in neutral or acid solution; thus the assignments for the neutral solutions cannot be made without further experiments. They were obtained as follows. 6*N*-NaOD solution was slowly added to an equal volume of a solution of the hydroxyproline in D<sub>2</sub>O, and the spectrum was recorded after each addition of a few drops of the NaOD. In this way, the peaks in the spectrum of the neutral solution could be followed stepwise as the spectrum continually changed with increasing pH, until the spectrum in 3*N*-alkali was obtained. This immediately relates the assignments for the alkaline solution with the known assignments for the neutral solution. The assignments for the allohydroxyproline spectrum were similarly obtained.

The proton chemical shifts and coupling constants, obtained from the analyses of the spectra and assigned in the above manner, are given in Tables 1 and 2. Also given are the results previously obtained for the neutral solutions, adjusted to the  $\tau'$  scale. [These were measured from a dioxan internal reference, assuming  $\tau$ (dioxan) 6.43. However, this is the  $\tau$  value for dioxan in carbon tetrachloride solution. The  $\tau'$  value in water solution is 6.26 (Table 3), and thus the  $\tau'$  values in Tables 1 and 2 for neutral solutions differ by 0.17 p.p.m.

TABLE 3.

Proton chemical shifts ( $\tau'$ ) of reference compounds in acid, neutral, and alkaline solution.

Sample	Acid	Neutral	Alkaline	$\tau'$ (D <sub>2</sub> O) - $\tau'$ (DCl)
2,2-Dimethyl-2-silapentane-5-sulphonate	10.000	10.000	10.000	0.000
t-Butyl alcohol.....	8.735	8.769	8.768	0.034
Acetonitrile .....	7.911	7.941	— *	0.030
Acetone .....	7.760	7.784	— *	0.024
Dimethyl sulphoxide .....	7.188	7.290	7.281	0.102
Tetramethylammonium bromide .....	6.795	6.822	6.825	0.027
Dioxan .....	6.238	6.257	6.253	0.019

\* Exchanges in alkaline solution.

from those previously given.] The  $\tau'$  values of some of the proposed internal reference compounds in acid, neutral, and alkaline solution are given in Table 3. The values for the neutral solution agree entirely with the unpublished results of Tiers.<sup>11</sup> Those in alkali are the same as those in neutral solution [with the possible exception of dimethyl sulphoxide, which is not a satisfactory internal reference (see later).] This demonstrates the suitability of any of these compounds as internal references in alkaline solution. However, the  $\tau'$  values in acid are not the same as those in neutral solution. That of dimethyl sulphoxide changes considerably, presumably owing to the formation of the sulphonium salt in acid solution.<sup>13</sup> Also, all the other  $\tau'$  values shift by *ca.* 0.030 p.p.m. to low field in acid solution. As these are quite different types of compound, the obvious implication of this constant low-field shift is that it is the DSS peak which moves upfield by 0.030 p.p.m. This has a simple explanation, in that in these strong-acid solutions (pH 0) the DSS will exist as the free sulphonic acid and not as the sulphonate ion (p*K* of benzenesulphonic acid, 0.707); this is supported by the result that increasing the acid concentration to 6*N* did not further affect the  $\tau'$  values. The shielding effect of the negative charge on the methyl groups could well be as much as 0.03 p.p.m. Thus, when an accurate comparison of the  $\tau'$  values for any compound in neutral and acid solution is required, 0.030 p.p.m. must be added to the values for the acid solution. Alternatively, the other internal reference compounds could be used. However, because the methyl groups of DSS absorb in a region of the spectrum which is transparent for almost all organic compounds, *i.e.*, it can be used as an internal reference for practically every organic compound, the  $\tau'$  scale will be used henceforth. The other reference compounds listed in Table 3 (except dimethyl sulphoxide) may be considered as secondary reference standards. The  $\tau'$  values given in

<sup>13</sup> Kiss and Foden, Paper presented at XIXth I.U.P.A.C. Congress, London, 1963, Abstracts A, p. 42.

Tables 1 and 2 demonstrate the pronounced influence of the electric charges on the various proton chemical shifts. In general, a positive charge would be expected to polarise the electrons of the C-H bonds so as to produce a low-field shift,<sup>14</sup> and *vice versa*. This agrees with the observed results. However, in the quantitative calculation of these shifts one has to consider other factors, such as the reaction field of each charge,<sup>15</sup> and these calculations will be given in a later publication.

*The Calculation of the Conformations of the Prolines in Acid and Alkaline Solution.*—The coupling constants of the cations (II) are the same as those of the corresponding zwitterions (I). It follows that the conformations are identical. This indeed would be expected on chemical grounds, as protonation of the carboxylate group would not affect the interactions which determine the molecular conformations. However, the coupling constants, and therefore the conformations, of the anions (III) of both molecules differ appreciably from those of the corresponding zwitterions, and it is these we wish to consider in detail. It is convenient at this point to discuss briefly the procedure and results of Parts I and II, which will be used here. The procedure adopted is based on the use of eqn. 1, which for the two coupling constants in a CH<sub>2</sub>-CHX fragment can be written as (assuming a dihedral angle of 120° between the methylene protons):

$$\begin{aligned} J_1 &= k_1 \cos^2 \phi - C \\ J_2 &= k_{1,2} \cos^2(120 \pm \phi) - C \end{aligned} \quad (2)$$

The values of  $k_1$ ,  $k_2$ , and  $C$  calculated for the zwitterions (I) were:  $C$ , zero;  $k_1$ , 10.5, 10.9, and 10.1 c./sec. for the C(2)-C(3), C(3)-C(4), and C(4)-C(5) fragments, respectively; and  $k_2$ , 13.7 c./sec. for the C(2)-C(3) fragment.<sup>1,6</sup> These constants would not be expected to change in the anions; <sup>4,5</sup> *e.g.*, the invariance of the C(4)-C(5) couplings (which should show the greatest deviations) is demonstrated by the CH<sub>2</sub>-CH coupling constant for  $\alpha$ -amino-n-butyric acid in acid and alkaline solution (6.07 and 6.1 c./sec., respectively <sup>16</sup>).

We are now in a position to consider the coupling constants of the anions (III). Inspection of Tables 1 and 2 shows one general trend. The vicinal coupling constants show a much greater variation in the zwitterions than in the corresponding anions. This result, when considered with the  $\cos^2 \phi$  law, immediately suggests that neither of the anions exists in a single rigid conformation. This may be proved rigorously as follows. When eqn. 2 is solved for the case of the C(3)-C(4) fragment in hydroxyproline, a value for  $k_1$  of 16.4 c./sec. is obtained, which is obviously much too large to be considered feasible. A similar result is obtained with the allo-isomer. Thus we can conclude definitely that neither of the anions exists in a single rigid conformation.

If the molecules exist as two or more rapidly interconverting conformers, the observed couplings will be the weighted mean of those of the distinct conformers. Also, if the percentages of the conformers change with temperature, so also will the observed spectra. In order to test this, the spectra of the alkaline solutions were recorded at 90°. The coupling constants obtained were the same, within the experimental error, as the corresponding ones at room temperature. Small changes in the chemical shifts occur, but these could well be due to hydrogen-bond cleavage or to changes in the reaction fields of the ions with temperature. Thus these results suggest that there is no change in the percentages of the conformers with temperature. There are two possible explanations of this result. Either the anions exist in one conformation, a result which we have already disproved, or the conformers have essentially equal energies and, therefore, assuming a small entropy difference between the conformers, equal populations.

This additional result makes it possible to calculate the distinct conformers from the observed coupling constants. The procedure adopted is as follows. We assume that the

<sup>14</sup> Buckingham, *Canad. J. Chem.*, 1960, **38**, 300.

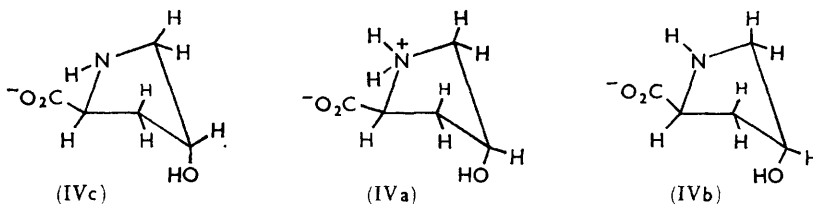
<sup>15</sup> Buckingham, Schaeffer, and Schneider, *J. Chem. Phys.*, 1960, **32**, 1227.

<sup>16</sup> Pachler, *Spectrochim. Acta*, 1963, **19**, 2085.



values of  $k_1$  and  $k_2$  found for the zwitterions apply to the anions, and furthermore that the ratio  $k_2 : k_1$  is constant for the different fragments of the ring. (As the values of  $k_1$  for the different fragments are so similar, we could alternatively have assumed that all  $k_2$  values are equal. However, the above is a more rigorous approximation.) This gives  $k_2 = 14.2$  c./sec. for the C(3)–C(4) and  $k_2 = 13.2$  c./sec. for the C(4)–C(5) fragments. These  $k$  values are inserted directly into equations of the form of eqn. 2, which now have  $n \cos^2 \phi$  terms, where  $n$  is the number of conformers. There are three such equations for each anion, making a total of six simultaneous linear equations in terms of the  $3n$  unknown  $\phi$  values (there are three dihedral angles for each conformer). Thus if we consider the simplest case of two distinct conformers, this gives six equations and six unknowns, and thus the dihedral angles can be obtained uniquely. The choice of the positive or negative signs in eqn. 2 gives eight possible sets of equations. In practice, only one of these sets for each compound gave a real solution. Even with this simplification, there are several possible combinations to consider. This is because, for each fragment, there will be two values of the dihedral angle (one for each conformer). Thus there are still four possible combinations of the dihedral angles to consider. These combinations must, however, satisfy the stringent condition that the dihedral angles are mutually consistent. This arises because only two dihedral angles are needed to define the conformation of a five-membered ring. The third value is thus a check on the particular combination considered. The values of the dihedral angles for the two symmetric conformations (the “ $C_2$ ” or “half-chair” and “ $C_s$ ” or “envelope” conformations<sup>17</sup>) have already been calculated as functions of the degree of buckle of the ring.<sup>1</sup> An extension of this calculation to include the many possible non-symmetric conformations becomes forbiddingly complex. This can be avoided by assuming that the non-symmetric conformations can be obtained by the linear combination of the symmetric modes. (This is of course analogous to normal vibrational-mode treatment.) It will be shown that, in practice, the conformations found only differ slightly from the symmetric modes. This is precisely the necessary condition for this “first order” approximation to be valid.

The results of these calculations are as follows. The two conformations of the hydroxyproline anion, (IVb) and (IVc), are both very similar to that of the zwitterion itself, (IVa), *i.e.*, they are the “envelope” conformations with C(4) projecting from the molecular plane so as to orient the hydroxyl group axially. They differ from the zwitterion conformation only in the angle of buckle of the ring. In the zwitterion it is  $54^\circ$ , whilst in the two conformations of the anion the angles of buckle are  $17^\circ$  and  $77^\circ$ , respectively. The coupling constants of the hydroxyproline anion, calculated on the basis of these two equally populous rapidly interconverting conformers, are:  $J_{2a-3a}$ , 7.9;  $J_{2a-3b}$ , 9.2;  $J_{3a-4b}$ , 2.5;  $J_{3b-4b}$ , 5.5;  $J_{4b-5a}$ , 2.3; and  $J_{4b-5b}$  5.1 c./sec. These are in very reasonable agreement with the observed values (7.1, 9.4, 2.4, 6.1, 3.0, and 5.3 c./sec., respectively).

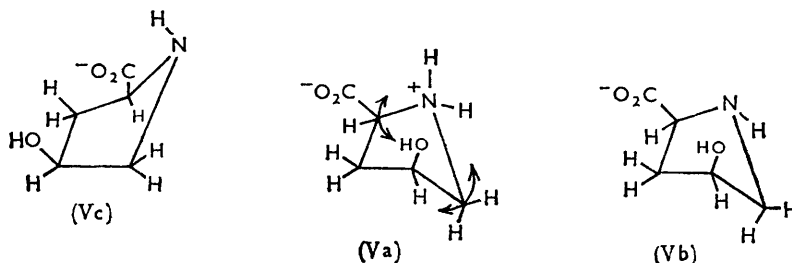


The two conformations of the allohydroxyproline anion are more difficult to describe, as neither of them is a symmetric conformation. They also may be considered as being derived from the zwitterion conformation, (Va), which is an “envelope” conformation with C(5) projecting from the molecular plane. The anion conformations are obtained from this by

<sup>17</sup> Kilpatrick, Pitzer, and Spitzer, *J. Amer. Chem. Soc.*, 1947, **69**, 2483.



displacing C(2) and C(5) in equal amounts in the directions shown. (This is equivalent to mixing a half-chair conformation.) One conformer, (Vb), is obtained by moving C(5)



down and C(2) up a small amount [shown by the short arrows in (Va)]. The other conformer, (Vc), is obtained by displacing C(5) and C(2) in the opposite directions, and in this case the extent of the displacement is much greater. This latter conformation approximates to the "envelope" conformation shown. Although these conformations can only be described roughly, they are defined precisely by the dihedral angles  $\omega_{12} - \omega_{51}$ <sup>18</sup> around the ring. These are, for the conformation of (Vb),  $-37$ ,  $+15$ ,  $-61$ ,  $+87$ , and  $-78^\circ$ , and, for the conformation of (Vc),  $+57$ ,  $-31$ ,  $-6$ ,  $+40$ , and  $-61^\circ$ . The coupling constants of the allohydroxyproline anion calculated from these conformers are:  $J_{2a-3a}$ , 8.8;  $J_{2a-3b}$ , 5.7;  $J_{3a-4a}$ , 6.7;  $J_{3b-4a}$ , 3.1;  $J_{4a-5a}$ , 3.0; and  $J_{4a-5b}$ , 4.9 c./sec. Again they are in reasonable agreement with the observed values (9.4, 6.2, 5.9, 3.4, 2.9, and 4.8 c./sec., respectively).

#### DISCUSSION

It can be seen that it is possible to explain the observed coupling constants of the two hydroxyproline anions on the basis of the conformers illustrated. Before discussing these conformers in more detail, it is convenient to consider the assumptions and approximations implied in the above treatment, especially as it is based on relatively small changes in the coupling constants. The experimental errors in the coupling constants have been estimated as  $\pm 0.1$  c./sec. The computational errors, including the approximations implicit in the ABX-type analysis, will be much less than this, in view of the large chemical-shift differences involved. The approximations involved in the use of eqns. 1 and 2 are more difficult to evaluate, but considerable theoretical<sup>2,19</sup> and experimental<sup>1,4,20</sup> evidence has accumulated which supports the  $\cos^2 \phi$  law. The assumptions of unchanged  $k$  values in acid and alkaline solution have already been considered, together with the estimation of  $k_2$  for the C(3)–C(4) and C(4)–C(5) fragments. The approximations involved are minimised by the shape of the  $\cos^2 \phi$  curve, which is very sensitive to small changes in  $\phi$  in the normal range of values of the dihedral angles ( $20$ – $80^\circ$ ). The most dubious assumption is that of equally populated conformers. If the observed coupling constants are precisely independent of temperature, then the energy differences between the rapidly interconverting conformers must be zero.

However, over the small temperature range observed (*ca.*  $60^\circ$ ), an energy difference of as much as 200 cal./mole would produce a change in the observed coupling of only 0.1 c./sec., which is well within the experimental error. This energy difference would make the ratios of the conformers 2 : 3 instead of 1 : 1 (assuming zero entropy difference between them), and would thus affect the quantitative conclusions reached. Consideration of all these assumptions shows that, although the general pattern which has emerged from these results is clear, *i.e.*, the spectra of the hydroxyproline anions are not consistent with any one conformation but are consistent with two rapidly interconverting conformers, the detailed quantitative aspects of these conclusions should be regarded with caution without

<sup>18</sup> Hendrickson, *J. Amer. Chem. Soc.*, 1961, **83**, 4537.

<sup>19</sup> Conroy, "Advances in Organic Chemistry," Interscience, New York, 1960, Vol. II, p. 311.

<sup>20</sup> Anet, *Canad. J. Chem.*, 1961, **39**, 789.

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further evidence. However, within these limitations, it is of interest to discuss the postulated conformations of the anions.

Inspection of the anion conformers (IV) and (V) shows that they are all zwitterion-like structures in which some of the ring atoms are slightly displaced, a displacement in one direction giving one conformer and in the reverse direction the other conformer. This is what would be expected, as the replacement of  $\text{NH}_2^+$  by NH would only slightly change the interactions which determine the molecular conformation, and the resulting deformation would follow the path of least resistance. In particular, the ring deformation of hydroxyproline could well be regarded as a very low-energy vibration. The highly strained structure of the allohydroxyproline zwitterion would preclude any considerable deformation in the direction of an increased angle of buckle. This is again what is observed; the displacement of the C(2) and C(5) atoms is much greater in the direction of decreasing angle of buckle (Vc) than in the opposite direction (Vb).

Thus the molecular changes which occur on the formation of the anions can be, at least qualitatively, rationalised. However, the central problem of the existence of two rapidly interconverting isomers in the anion, as distinct from the zwitterion, has yet to be answered. We suggest that this is due to the increased possibility, in the anion, of isomerism about the nitrogen atom. Clearly, in any conformation of the anion there will be two distinct isomers, owing to the inversion at the nitrogen atom. Such inversion is known to be too fast to be observed by nuclear magnetic resonance techniques,<sup>21</sup> except in the special case of the aziridines, in which the spectra of the distinct isomers can be obtained.<sup>22,23</sup> We suggest that each of the anion conformations shown corresponds to the two possible positions of the N-H proton in each molecule. It may be thought surprising, at first sight, that such a small change as that of the position of the N-H proton could give rise to an appreciable change in the molecular conformation. However, the different possibilities of intramolecular hydrogen bonding in the two cases could provide sufficient interaction energy for this purpose. In particular, the increased angle of buckle of one hydroxyproline conformer, (IVb), could well be due to hydrogen bonding between the hydroxyl group and the nitrogen lone-pair, analogous to the well-known intramolecular hydrogen bonds in 5-hydroxy-1,3-dioxans.<sup>24</sup> Similar hydrogen-bonding possibilities exist in the allo-forms, and it is on this basis that the position of the N-H proton has been assigned in the particular conformers. The existence of intramolecular hydrogen bonds in water solution is well known in the nucleic acid field.<sup>25</sup> Also, in a recent study of the effect of solvents on the rotational isomerism of 2-methoxyethanol,<sup>5</sup> water behaved in precisely the same manner as carbon tetrachloride, whereas acetone, in which solvent-solvent interactions are negligible, was much more effective in changing the percentage of the rotational isomers, presumably by breaking the intramolecular hydrogen bonds. Thus the formation of intramolecular hydrogen bonds in aqueous solution may well be a considerable factor in determining the conformations of compounds in which strong intramolecular hydrogen bonding is possible.

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<sup>21</sup> Kincaid and Henriques, *J. Amer. Chem. Soc.*, 1940, **62**, 1474.

<sup>22</sup> Heesch, Thesis, University of Illinois, 1959.

<sup>23</sup> Loewenstein, Neumer, and Roberts, *J. Amer. Chem. Soc.*, 1960, **82**, 3599.

<sup>24</sup> Brimacombe, Foster, and Haines, *J.*, 1960, 2582.

<sup>25</sup> Michelson, *Ann. Reports*, 1960, **57**, 294.