686. *An Infrared and Nuclear Magnetic Resonance Study of the Nature of Ethylenediaminetetra-acetic Acid and Some Related Substances in Solution: Hydrogen Bonding in α-Amino-polycarboxylic Acid Systems.*

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Ionisation patterns for a number of α-amino-polycarboxylic acids in water are discussed with the aid of infrared and nuclear magnetic resonance spectral data. Systems containing intramolecular hydrogen bonds are suggested to explain certain prominent features of the spectra. Examples have been found of substances which change their structure, from a non-zwitter-ion to a zwitter-ion form, when the crystals are dissolved in water.

ETHYLENEDIAMINETETRA-ACETIC ACID (EDTA) (represented as H_4Y) is a multifunctional α-amino-acid, which, on the basis of pK measurements of its dilute aqueous solution, Schwarzenbach and Ackermann [1] have concluded behaves as a double zwitter-ion. However, the solid-state infrared spectra of the acid and its di- and tetra-sodium salts have been interpreted as showing that, in the solid state, the acid and the disodium salt are not in a zwitter-ion form.[2] The dihydrochloride, H_6YCl_2, has been prepared by Olsen and Margerum [3] who have measured the pK values for the first three acid dissociations of this compound. They consider that their measurements, in conjunction with those of Schwarzenbach and Ackermann,[2] indicate unequivocally that the acid exists and ionises as a zwitter-ion, but they do not attempt to explain the spectral data. The two alternative ionisation patterns for the acid are set out by Olsen and Margerum.[3]

TABLE 1.

Compounds examined, and methods used.

Each number represents the number of protons added to the " bare " anion of the appropriate amino-acid of the form $NRR'{\cdot}CH_2{\cdot}CO_2^-$ (R and R′ are as indicated below), or to the neutral molecules for amines.

Substance	Nuclear magnetic resonance	Infrared bands		
		Solid	In D_2O	In H_2O
EDTA	6,4*,2,1,0	6,4,2,1,0	4,2,1,0	6,2,1,0
NTA	4,3*,1,0	4,3,1,0	3,1,0	4,1,0
MIDA	3,2,1,0	3,2,1,0	2,1,0	3,1,0
IDA	3,2,1,0	3,2,1,0	2,1,0	3,1,0
en	2,1,0			2,1,0
gly	2,1,0	2,1,0	1,0	2
DMG	2,1,0	2,1,0	1,0	2
NEt_3				1,0
$N(CH_2{\cdot}CH_2{\cdot}OH)_3$				1,0
TMEDA	2,1,0			
Betaine	1,0	1,0	1,0	
NTA-betaine	3,0		2,0	

* Supersaturated solution.

Abbreviations for substances used in this Table and elsewhere.

EDTA = Ethylenediaminetetra-acetic acid, $[(HO_2C{\cdot}CH_2)_2N{\cdot}CH_2{\cdot}]_2 = H_4Y$.
NTA = Nitrilotriacetic acid, $N(CH_2{\cdot}CO_2H)_3 = H_3X$.
MIDA = *N*-Methyliminodiacetic acid, $Me{\cdot}N(CH_2{\cdot}CO_2H)_2 = H_2Z$.
IDA = Iminodiacetic acid, $NH(CH_2{\cdot}CO_2H)_2 = H_2W$.
gly = Glycine, $NH_2{\cdot}CH_2{\cdot}CO_2H$.
DMG = *NN*-Dimethylglycine, $NMe_2{\cdot}CH_2{\cdot}CO_2H$.
en = Ethylenediamine, $NH_2{\cdot}CH_2{\cdot}CH_2{\cdot}NH_2$.
TMEDA = Tetramethylethylenediamine, $NMe_2{\cdot}CH_2{\cdot}CH_2{\cdot}NMe_2{\cdot}$.
Betaine = $^+NMe_3{\cdot}CH_2{\cdot}CO_2^-$.
NTA-betaine = $^+NMe(CH_2{\cdot}CO_2H)_2{\cdot}CH_2{\cdot}CO_2^-$.

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[1] Schwarzenbach and Ackermann, *Helv. Chim. Acta*, 1947, **30**, 1798.
[2] Chapman, *J.*, 1955, 1766.
[3] Olsen and Margerum, *J. Amer. Chem. Soc.*, 1960, **82**, 5602.

In the present investigation a number of α-amino-polycarboxylic acids, including ethylenediaminetetra-acetic acid, and their various salts have been examined in the solid state, in solution in heavy water and in water, by infrared spectroscopy [4] combined with nuclear magnetic resonance measurements on aqueous solutions.

A number of model compounds, of the types $NR_2{\cdot}CH_2{\cdot}CO_2H$, R_3N, $NR_2{\cdot}CH_2{\cdot}CH_2{\cdot}NR_2$, have also been examined. All the compounds examined are listed in Table 1, together with the abbreviations and symbols used in this paper.

EXPERIMENTAL

Infrared Spectra.—These were taken mainly on a Perkin-Elmer 21 instrument equipped with scale expander, and sodium chloride or fluorite prisms. Crystal spectra were obtained for Nujol or hexachlorobutadiene mulls between sodium chloride plates. Spectra of solutions in water and heavy water were recorded for capillary films between fluorite plates. The spectra of the very dilute saturated solutions of ethylenediaminetetra-acetic acid and nitrilotriacetic acid in heavy water were recorded by using a barium fluoride cell (thickness 0·025 mm.) with a similar cell of heavy water in the reference beam. For the latter spectra the scale expander was used at " ×5 " expansion. These two acids were also examined in 0·01M- and 0·05M-deuterium chloride in heavy water: the spectra were weaker, owing to decreased solubility,[5] but band positions were identical with those in heavy water. Quantitative spectra of some heavy water solutions were recorded by using the barium fluoride cells.

The spectra of water solutions (Table 3) were obtained on a Perkin-Elmer 137 " Infracord " instrument, for capillary films between silver chloride plates, with gauze filters in the reference beam.

Crystal spectra were generally recorded of crystals as prepared and dried over phosphorus pentoxide at atmospheric pressure for a few hours, and of samples dried over this desiccant *in vacuo* at 110°.* All the hydrochlorides were dried either *in vacuo* or in dry hydrogen chloride, without heating.

Infrared spectral results are in Tables 2 and 3.

Nuclear Magnetic Resonance (*n.m.r.*) *Spectra*.—The majority of these were obtained at 40 Mc./sec. by using a Varian Associates V4300B spectrometer and 12″ electromagnet with flux stabilisation and, except for a few cases, sample spinning. Positions of references are quoted as chemical shifts on the τ scale ($SiMe_4 = 10{\cdot}00$) and have been measured against t-butyl alcohol as an internal reference by using side-bands generated by a Muirhead-Wigan D695A decade oscillator: on this scale the τ value of t-butyl alcohol is 8·78 p.p.m.

A Perkin-Elmer 40 Mc./sec. spectrometer was used for some of the measurements; certain compounds were examined on both instruments, and no significant differences between the pairs of spectra were observed.

Some spectra were obtained for non-spinning samples of heavily supersaturated solutions (see Table 4). These solutions were obtained by mixing the calculated amounts of aqueous solutions of the disodium salts with dilute hydrochloric acid, so that the final solution was about 0·25M in, for example, ethylenediaminetetra-acetic acid, H_4Y. To maintain supersaturation, the solutions were filtered before mixing, all vessels were thoroughly cleaned before use, all glass surfaces, particularly the tip of the pipette used to transfer the liquid into the spectrometer sample tube, were kept free from scratches by " flame polishing," and all glass surfaces were wetted with distilled water before the supersaturated solution was allowed to touch them. In this way about one of each three attempts to obtain spectra before the acid was precipitated were successful.

Attempts to obtain the spectrum of the saturated acid solutions in water by using the 15 mm. probe on the Varian spectrometer were unsuccessful, as was an attempt to obtain the spectrum of ethylenediaminetetra-acetic [^{2}H]acid, D_4Y, in heavy water, saturated at about 70°, with this probe: no signal could be found. A supersaturated solution (0·025M) of the

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* Sawyer and Paulsen [6] report a peak at 1675 cm.$^{-1}$ in several spectra, but Chapman [2] has shown that this is due to water molecules in the crystal lattice: this observation has been confirmed here by drying in this way.

[4] Gore, Barnes, and Petersen, *Analyt. Chem.*, 1949, **21**, 382.

[5] Palei and Udal'tsova, *Russ. J. Inorg. Chem.*, 1960, **5**, 1121.

[6] Sawyer and Paulsen, *J. Amer. Chem. Soc.*, 1958, **80**, 1597.

TABLE 2.

Wave numbers (cm.$^{-1}$) of principal bands in the 1350—1750 cm.$^{-1}$ region of the infra-red spectra. (n = no. of protons per molecule, added to the bare anion; cf. heading to Table 1. For abbreviations see Table 1.)

		Solution				Crystal			
	n	CO_2H	CO_2^-(as)	CO_2^-(sym)	Δ	CO_2H	CO_2^-(as)	CO_2^-(sym)	Δ
MIDA	0		1586	1410	176		1592	1416	176
	1		1628	1406	222		1625	1400	225
	2	1730	1631	1408	223	1692			
						1735			
	3	1734				1724			
						1761			
EDTA *	0		1585	1414	171		1597	1415	182
	1		1585	1406	179		1596	1409	187
			1625		219		1625sh		216
	2		1628	1400	228		1628	1400	228
							1658sh		
	4	1725	1626	1410	216	1695			
	6	1715				1736			
	0		1588	1409	179		1598	1409	189
	1		1625	1400	225		1632	1361	237
							1673	1395	312
NTA	2	1730	1635	1406	229	1732	1635		
							1665		
	3	1726	1630	1405	225	1736			
	4	1732				1713			
						1734			
						1764			
IDA	0		1583	1410	173		1588	1425	163
	1		1618	1408	210		1625	1408	217
	2	1727	1624	1406	218	1712	1587	1399	188
	3	1728				1727			
						1760			
DMG	0		1582	1410	172		1603	1408	195
	1		1629	1401	228		1631	1396	235
	2					1746			
gly	0		1582	1413	169		1579	1415	164
	1		1615	1414	211		1600	1415	185
	2	1745				1720			
Betaine	0		1627	1407	220				
	1	1735							
NTA-betaine	0		1620	1402	218				

sh = shoulder. Δ = difference between frequencies of the asymmetric and symmetric vibration frequencies.

* The peak reported [2] at 1668 cm.$^{-1}$ in the crystal spectrum of disodium dihydrogen ethylenediaminetetra-acetate was observed as a prominent shoulder at 1658 cm.$^{-1}$: use of the fluorite prism failed to resolve this as a separate peak.

TABLE 3.

Infrared spectra: aqueous solutions in 1000—1200 cm.$^{-1}$ region. (Values in italics indicate the peak assigned to C–N stretching vibration.)

n	MIDA	EDTA	NTA	IDA	NEt_3	$N(CH_2{\cdot}CH_2{\cdot}OH)_3$
0		990m			1000w	1025s
	1040m	1010sh	990m	1040m	1020m	1070s
	*1120*m	1025sh	1010sh	1140s	*1075*s	1120w
	1200w	*1120*s	*1140*m	1210w	1095m	*1150*m
					1140m	
1	1050w	990m	1060w	1060m	1020m	1005m
	1110w	1010sh	1075w		1040m	1030w
	1155w	1030sh			1070m	1065s
		*1120*s			1160m	1095s
2	V. weak	V. weak				

deuterated acid in heavy water was prepared by dissolving the appropriate quantity of disodium ethylenediaminetetra-acetate in heavy water in a 15 mm. sample tube and adding the calculated quantity of concentrated (~5M) hydrochloric acid with an Agla micrometer syringe. In this way only a small amount of ordinary water was added to the heavy water, and the CH_2 spectrum was not obscured by the HOD absorption. Field inhomogeneities were such that the doublet spectrum became a single broad peak at the side of the HOD peak.

Results are in Table 4.

TABLE 4.

Nuclear magnetic resonance spectra of aqueous solutions: proton resonances in p.p.m. on the τ scale ($SiMe_4$ = 10·00).

	MIDA		EDTA †		NTA	IDA	Betaine		NTA-betaine	
n	CH_3	CH_2	Inner CH_2	Outer CH_2	CH_2	CH_2	CH_3	CH_2	CH_3	CH_2
0	7·78	6·98	7·55	6·98	6·89	6·65	6·72	6·06	6·56	5·62
1	7·11	6·28	7·21	6·73	6·22	6·46	6·66	5·66		
2	7·01	6·03	6·35	6·13	6·01	5·14				
3	6·91	5·76			5·82 *	4·90			5·33	5·10
4			6·53 *	6·00 *	4·83					
6			6·01	5·58						

	DMG		TMEDA		gly	en ‡
n	CH_3	CH_2	CH_3	CH_2	CH_2	CH_2
0	7·78	7·05	7·50	7·10	6·81	7·37
1	7·10	6·25	7·28	6·78	6·41	6·98
2	6·99	5·86	6·99	6·33	6·15	6·58

* Determined on supersaturated solution.

† The spectra of the ethylenediaminetetra-acetate ions have two lines in the intensity ratio 1 : 2; the column labelled " inner " has the lower intensity. In the spectra of *N*-methyliminodiacetic acid the two lines observed have intensities in the ratio 3 : 4. The CH_3 column gives shifts for the less intense line. Assignments for *NN*-dimethylglycine and tetramethylethylenediamine were made similarly.

‡ In very acid (6M-hydrochloric acid) solution, the spectrum also shows a poorly resolved triplet, with intensity ratios 1 : 2 : 1. The centre of the triplet has τ 2·11 p.p.m., the separation between the outer members being ~1·7 p.p.m.

Preparations.—Analyses were carried out by standard micro-techniques for carbon, hydrogen, and nitrogen. Chloride was determined by electrometric titration against standard silver nitrate. Where appropriate, pH titrations and complexometric titrations were also carried out. All analyses were satisfactory. Acids and their sodium salts were dried at 110° over phosphorus pentoxide unless otherwise stated.

Deuterium chloride solution. This solution (0·01M and 0·05M) was prepared from 5M-hydrochloric acid and the calculated excess of heavy water. Since the infrared absorption of HOD is well removed from the region examined, the addition of 1% of H_2O to D_2O does not affect the infrared spectra significantly.

Ethylenediaminetetra-acetic acid, EDTA. The free acid was prepared by acidifying a solution of the " AnalaR " disodium salt in water with hydrochloric acid and recrystallising the acid twice from a large volume of water. The dihydrochloride, H_6YCl_2, was prepared by dissolving the acid in hot " AnalaR " 6M-hydrochloric acid; the crystals that separated were recrystallised twice from " AnalaR " 6M-hydrochloric acid and dried in an atmosphere containing dry hydrogen chloride (aluminium trichloride–phosphorus pentoxide as drying agent). The tetrasodium salt was prepared by precipitation from aqueous solution by ethanol. The trisodium salt was prepared by dissolving the disodium salt in slightly more than the requisite amount of hot sodium hydroxide solution, adding about three times the volume of methanol, and then sufficient ethanol to initiate precipitation. This procedure eliminates separation into two liquid phases which occurred when ethanol alone was used as precipitant.

Solutions of mixtures of di-, tri-, and tetra-sodium salts were prepared by weighing appropriate mixtures (1 : 0, 3 : 1, 1 : 1, 1 : 3, 0 : 1) of the di- and tetra-sodium salts and dissolving these in heavy water.

Solutions of tetra- and tri-sodium salts and the dihydrochloride, H_6YCl_2, for n.m.r. work were prepared by adding the appropriate amounts of sodium hydroxide or hydrochloric acid to solutions of the disodium salt.

Nitrilotriacetic acid, NTA. Hopkin and Williams G.P.R. acid (H_3X) was twice recrystallised from a large volume of water. The hydrochloride, H_4XCl, was prepared in the same way as that of ethylenediaminetetra-acetic acid. The preparation and purification of sodium salts also followed the procedures used for ethylenediaminetetra-acetic acid.

Methyliminodiacetic acid, MIDA. Hopkin and Williams G.P.R. methyliminodiacetic acid was used without purification, beyond drying *in vacuo* at 110°. Sodium salts were prepared in the way described for ethylenediaminetetra-acetic acid.

Iminodiacetic acid, IDA. All the substances were prepared, in the same way as the *N*-methyliminodiacetic acid analogues, from Hopkin and Williams G.P.R. acid.

Glycine. "AnalaR" glycine was used without purification. The sodium and hydrochloride salts were prepared by crystallising glycine from alkaline or acid solutions.

Dimethylglycine, DMG. The B.D.H. hydrochloride was used without purification. The sodium salt was prepared by adding alcohol to a mixture of the hydrochloride and sodium hydroxide solutions in the appropriate concentrations. It was recrystallised from alcohol (98%). *NN*-Dimethylglycine is very soluble in both alcohol and water and was prepared by passing carbon dioxide into an alcoholic solution of the sodium salt. The precipitated sodium carbonate was filtered off, and the alcoholic solution of *NN*-dimethylglycine was evaporated. The substance recrystallised from dioxan.

Ethylenediamine hydrochloride. This was recrystallised three times from water.

Solutions for infrared and n.m.r. experiments were made up by dissolving the crystals in M-hydrochloric acid or in appropriate amounts of sodium hydroxide solution.

Tetramethylethylenediamine, TMEDA. Rohm and Haas reagent (70%) was distilled in a vacuum, dissolved in "AnalaR" concentrated hydrochloric acid, and evaporated to crystallisation. The crystals were twice recrystallised from a **3** : 1 mixture of 98% alcohol and "AnalaR" concentrated hydrochloric acid.

Solutions for n.m.r. work were made up in the same way as the ethylenediamine solutions.

Betaine and NTA-betaine. Betaine hydrochloride (B.D.H. reagent) was used as starting material. The betaine was prepared by treatment of this with sodium hydroxide solution followed by crystallisation.

The derived betaine was prepared by methylation of sodium nitrilotriacetate in an excess of sodium hydroxide solution with dimethyl sulphate. The compound was isolated by evaporation to small bulk; the sodium sulphate crystallised, and addition of dilute acid precipitated a compound $[^+NMe(CH_2{\cdot}CO_2^-)_3]H_4Na_2$. This was twice recrystallised from water. Solutions for n.m.r. spectroscopy were prepared by dissolving it in an excess of acid or alkali; those for infrared spectroscopy by dissolution in an excess of sodium deuteroxide.

DISCUSSION

Results of p*K* measurements are normally used to interpret ionisation patterns: pK data are available[7] for all the compounds examined, and detailed comparison with a spectroscopic approach is possible. Any method for investigating ionisation patterns must be capable of either (*a*) locating the proton directly or (*b*) detecting its influence or the effect of its loss on the parts of the molecule adjacent to its point of attachment. Infrared and nuclear magnetic resonance (n.m.r.) methods will do this. High-resolution n.m.r. measurements are easily made on aqueous solutions, but there are some difficulties in the infrared region due to the very high absorption by water at some frequencies. These may be reduced[4] by working also with heavy water solutions, since the regions of high absorption are different for the two solvents. Changing from ordinary to heavy water changes the nature of a solute molecule slightly, in that exchangeable hydrogen is exchanged for deuterium. It is reasonable to conclude, however, that there will be no gross structural change of the solute molecule when the solvent is changed in this way (cf. ref. 8).

For the aqueous systems studied here in the infrared region it is not possible to detect the absorption bands due to the NH, ND, OH, or OD groups owing to the strong broad

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[7] Schwarzenbach and Sillén, "Stability Constants. Part I. Organic Ligands," *Chem. Soc. Special Publ. No.* 12, 1957.

[8] Dodd, Miller, and Wynne-Jones, *J.*, 1961, 2790.

absorption bands of H_2O and D_2O. On the time scale of n.m.r. spectroscopy the lines due to protons attached to nitrogen or oxygen coalesce with the water line owing to rapid exchange with the solvent.[9]

For solid-state infrared spectra unambiguous assignment of OH or NH vibrations is difficult for the substances examined. Solid-state spectra have been recorded for comparison with the regions investigated in solution * and with previously reported spectra,[2,6] but no attempt has been made to investigate the O–H, N–H region in detail.

Infrared Spectra.—(i) *Heavy-water solutions:* CO_2H *and* CO_2^- *vibrations.* The most characteristic absorptions in the infrared spectrum of carboxylic acids and their salts are those due to the stretching frequency of the carbonyl group of CO_2H or the antisymmetric (as) stretching frequency of CO_2^-. These bands occur in a region of the spectrum (1750—1550 cm.$^{-1}$) in which water absorbs strongly but heavy water does not. The change in frequency of the (as) absorption on protonation of a carboxylate group is so large (~140 cm.$^{-1}$) that this is by far the best diagnostic test of whether or not a proton is associated with a carboxylate group.

A hydrogen-bonded carboxylic acid group has a lower infrared absorption frequency than a free carboxylic acid group [10] and the antisymmetric vibration frequency of the carboxylate group increases when hydrogen bonding is present.†,[11c] The antisymmetric frequency of a strongly hydrogen-bonded CO_2^- group is uniformly about 35 cm.$^{-1}$ higher than that of a non-hydrogen-bonded CO_2^- group; the frequency of a strongly hydrogen-bonded CO_2H group is about 30 cm.$^{-1}$ lower than that of a " free " CO_2H group.

The frequencies of these groups depend upon the class of compound. For α-amino-acids the values (CO_2H 1730 cm.$^{-1}$, CO_2^- 1680 cm.$^{-1}$) are higher than for the related succinic acids (CO_2H 1680 cm.$^{-1}$, CO_2^- 1550 cm.$^{-1}$). The changes from compound to compound within a class are less (~10 cm.$^{-1}$) but are detectable, and it is therefore necessary, for a given acid, to have the spectra of both the non-protonated and the fully protonated form.

Investigations of various amino-acid–metal complexes have shown [6,13,14] that, as the covalency of the $M-O_2C$ bond increases, the antisymmetric frequency increase; and the symmetric frequency decreases. The difference between the two is suggested [6,14] as a measure of the covalency of the M–O bond. Small shifts of the symmetric frequency to lower values have been observed in our work when hydrogen bonding occurs.

The ionisation patterns of the substances examined can be deduced from the infrared spectra of the various salts in solution in this region. Some finer details of structure, particularly the presence or absence of hydrogen bonding, need careful examination of the detailed frequency variations between the spectra of the various protonated forms.

(ii) *Water solutions:* C–N *region.* The C–N assignment is by no means certain and several compounds containing CH_2–N or CH_3–N groups were therefore examined as models These compounds and the spectra observed in the 1000—1200 cm.$^{-1}$ region are set out in Table 3. Sawyer and Paulsen,[6] in their examination of the solid tetra-, tri-, and di-sodium salts of ethylenediaminetetra-acetic acid, observed a band at 1130 cm.$^{-1}$ which they assigned to the C–N vibration.

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* The infrared spectra obtained from crystals are clearly those of one species, whilst solutions contain various protonated forms in equilibrium. However, in all the cases reported here it may be shown by calculation from the known p*K* values [7] that the solutions contain essentially one form (concentration ~95%).

† A case [12] ($CF_3\cdot CO_2Na,2CF_3\cdot CO_2H$) in which the (as) frequency is lowered is exceptional in that both atoms of the CO_2^- group are thought to be hydrogen-bonded. A symmetrical influence upon the two oxygen atoms causes the symmetric and antisymmetric frequencies to move in the same direction.[13a]

[9] Pople, Schneider, and Bernstein, " High-resolution Nuclear Magnetic Resonance," McGraw-Hill, London, 1959, pp. 246, 442 *et seq.*

[10] Bellamy, " The Infra-red Spectra of Complex Molecules," 2nd edn., Methuen, London, 1958.

[11] Eberson, *Acta Chem. Scand.*, 1959, **13**, (*a*) 203, (*b*) 211, (*c*) 224.

[12] Klemperer and Pimentel, *J. Chem. Phys.*, 1954, **22**, 1399.

[13] Nakamoto, Morimoto, and Martell, *J. Amer. Chem. Soc.*, (*a*) 1961, **83**, 4528, (*b*) 1962, **84**, 2081.

[14] Sawyer and Paulsen, *J. Amer. Chem. Soc.*, 1959, **81**, 816; Sawyer and McKinnie, *ibid.*, 1960, **82**, 4191.

Table 3 shows that all the compounds examined have a strong absorption in the region 1050—1150 cm.$^{-1}$ when the N atom is " free." This absorption disappears on protonation of the N-atom. No band can be found in the spectra of the protonated compounds which can be assigned to the grouping $C-NH^+$.

Although the assignment of this band to the C–N vibration is probably correct, this is not general; *e.g.*, the spectrum of sodium salt of glycine in water [4] shows no strong absorption in this region. However, within a restricted class of compounds such as the α-amino-polycarboxylic acids, the band is prominent and has a characteristic position at about 1120 cm.$^{-1}$ in solution.

(iii) *Solid-state spectra: structural changes on crystallisation from solution.* In the solid state there are numerous possibilities for intermolecular hydrogen bonding, and many of the substances which exist as zwitter-ions can exist in several crystalline modifications (*e.g.*, glycine in three and ethylenediaminetetra-acetic acid in two [15]), though the modifications have similar spectra in the $CO_2H-CO_2^-$ region. Table 2 shows that certain substances have very different crystal and solution spectra: these are the free acids, ethylenediaminetetra-acetic (H_4Y), methyliminodiacetic (H_2Z), and nitrilotriacetic (H_3X) acid. These are examined in detail below, where it is shown that in solution these substances exist as hydrogen-bonded zwitter-ions,* but in the solid as normal carboxylic acids.

Nuclear Magnetic Resonance Measurements.—Model compounds. Protonation produces changes in the spectra of all the compounds examined (cf. refs. 17 and 18). The hydrogen atoms of the CH_2 and CH_3 group show a systematic change in chemical shift. Proton addition localises electrons to bind the proton and causes a lowering of electron density, hence a lowering of shielding effects and systematic decreases in chemical shift. Such decreases [17,18] are observed (see Table 4).

The basis of the interpretation of the n.m.r. data is to examine the chemical shifts of the groups adjacent to the nitrogen and the carboxylate group undergoing protonation. This is done with model compounds whose ionisation patterns are considered to be known unequivocally. The ionisation data for the acids under examination are then deduced by comparison. It is important that the models should have structures closely analogous to those under examination. Glycine and ethylenediamine were considered as models, but, as seen from Table 4, they give values of $\Delta\tau$ which are too small for their use as good analogues.

Interpretations are therefore based upon the n.m.r. data for dimethylglycine. Here we assume that the first new proton becomes attached to the nitrogen atom and this is supported by pK and infrared data. The n.m.r. data show that, on addition of the first proton, $\Delta\tau$ for both CH_3 and CH_2 groups is quite large (CH_3 0·68, CH_2 0·80 p.p.m.). A shift of both by a roughly similar amount is to be expected since both are bound directly to the nitrogen atom undergoing protonation.

When the second proton is added, $\Delta\tau$ for CH_2 is 0·39 and for CH_3 0·11 p.p.m. Since the CH_2 is separated from the H of CO_2H by two atoms, it is to be expected that $\Delta\tau$ for this protonation will be smaller than for the first, where only one atom, N, separates the

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* Throughout this paper the term " hydrogen bond " used without qualification is taken to include any direct interaction between the $\geqslant NH^+$ and the carboxylate group. Although there may be a distinction mechanistically between an electrostatic interaction in such a system and a hydrogen bond with some covalent character, stereochemically the interactions detected are equivalent to hydrogen-bond formation. The spectroscopic techniques used reveal the existence of such direct interactions whether these are of purely electrostatic origin (cf. our data on the betaines in which only an electrostatic effect can operate) or involve a hydrogen bond with, possibly, some covalent character. In any event the whole question of electrostatic and covalent function in the hydrogen bond is not finally settled.[16] The term " normal acid " in this paper denotes a non-zwitterionic form.

[15] Leblanc and Spell, *J. Phys. Chem.*, 1960, **64**, 949.

[16] Pimentel and McClellan, " The Hydrogen Bond," Freeman, London, 1960.

[17] Loewenstein and Roberts, *J. Amer. Chem. Soc.*, 1960, **82**, 2705.

[18] Grunwald, Loewenstein, and Meiboom, *J. Chem. Phys.*, 1957, **27**, 641.

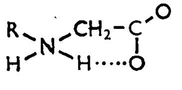
proton and the CH_2. There is an observable $\Delta\tau$ for the CH_3 protons on the second proton addition. We ascribe this effect to the breaking of a ring-hydrogen bond (see below).

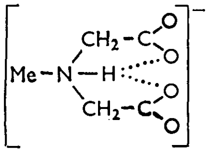
It is to be expected by analogy with dimethylglycine that in protonation of the other compounds addition of H^+ to N will cause τ to change by 0·7—0·8 p.p.m. for the groups attached directly to N. Addition of a proton to a CO_2^- group should give a $\Delta\tau$ for the adjacent CH_2 of about 0·4 p.p.m. The protonation of dimethylglycine is complete when two protons have been added, whilst with polycarboxylic amino acids the addition of the first two protons leaves some CO_2^- groups unprotonated. The protons in the solution, will exchange rapidly with the carboxylate groups and the CH_2 signals will be averaged.

Hydrogen Bonding and Ionisation Patterns of α-Amino-polycarboxylic Acids.—Intermolecular hydrogen bonding occurs if there is strong association of the molecules or ions. Such association is most likely for the uncharged molecules, since coulombic repulsion will tend to keep similarly charged ions apart. Of the uncharged molecules discussed here glycine will be most likely to associate, since the others will be kept further apart by steric hindrance of the substituting groups. Accurate freezing-point data [19] for glycine indicate that at a concentration of 3·90% (that of the solution used to obtain the infrared spectrum) the degree of association is less than 5·5%. All the other solutions examined were of lower concentration. The other compounds are less likely than glycine to be associated and thus the spectral effects observed cannot be accounted for in terms of intermolecular hydrogen bonding.

For all the substances examined, p*K* data [9] indicate that the first proton is added to the nitrogen atom of the unprotonated anion. In all cases (except the ethylenediaminetetra-acetate ion, discussed below) the first proton raises the CO_2^- frequency by ~30 cm.$^{-1}$ (Table 2). A similar shift has been reported for alanine (ref. 10, p. 241). We suggest that this shift is due to quite strong intramolecular interaction, probably hydrogen bonding.

It is noteworthy that even in forming an analogue where hydrogen bonding is impossible (*viz.*, a betaine) there may be frequency changes similar to those observed on protonation of the anion. This is illustrated by the data for dimethylglycine and nitrilotriacetic acid (Table 2). The CO_2^- frequency change in going from $NMe_2{\cdot}CH_2CO_2^-$ to $^+NHMe_2{\cdot}CH_2{\cdot}CO_2^-$ is 39 cm.$^{-1}$, from $NMe_2{\cdot}CH_2{\cdot}CO_2^-$ to $^+NMe_3{\cdot}CH_2{\cdot}CO_2^-$ 37 cm.$^{-1}$, from $N(CH_2{\cdot}CO_2^-)_3$ to $^+HN(CH_2{\cdot}CO_2^-)_3$ 39 cm.$^{-1}$, and from $N(CH_2{\cdot}CO_2^-)_3$ to $^+NMe(CH_2{\cdot}CO_2^-)_3$ 32 cm.$^{-1}$. Addition of H^+ or Me^+ to nitrogen produces the same effect on the frequencies and a direct (electrostatic) interaction of the CO_2^- group with the positive centre is a likely origin of this effect. (We have already discussed whether the interaction is inductive in nature or direct and have presented arguments in favour of the direct interaction.[20]) Spectroscopically, direct interaction of this sort and " hydrogen bonding " are indistinguishable but the interaction is stereochemically equivalent to a hydrogen bond [20] and for the simple amino-acids a hydrogen-bonded ring structure (I) is consistent with the spectral results.


(I)


(II)

The geometry of such a ring as (I) is unusual: if normal valency angles and distances are assumed,[21] it is most likely that the atoms N, H, O are not collinear (cf. ref. 16, p. 101). The CO_2^- vibration frequency of the sodium salt of alanine increases by 48 cm.$^{-1}$ on protonation of the nitrogen (ref. 10, p. 241), but β-alanine shows no change at all. It appears that in such systems a 5-membered hydrogen-bonded ring is more stable than a

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[19] Roth, *Z. phys. Chem.*, 1903, **43**, 539.
[20] Chapman, Lloyd, and Prince, *Proc. Chem. Soc.*, 1962, 336.
[21] L. E. Sutton *et al.*, " Interatomic Distances," *Chem. Soc. Special Publ. No.* **13**, **1958**.

6-membered ring. Other examples of 5-membered rings are given in ref. 16, pp. 170, 171, 174.

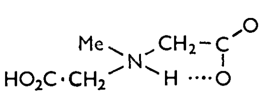
The differences in frequency of the antisymmetric and symmetric carboxylate vibrations (Table 2) are considerably higher for the protonated than for the non-protonated forms. This is consistent with strong interaction of the hydrogen-bonding type. Other data[14] suggest that the hydrogen-bonded ring may be quite common for α-amino-acids in solution. These differences are about 210 cm.$^{-1}$. This contrasts with the value (173 cm.$^{-1}$) for β-alanine which does not form a hydrogen-bonded ring.

With amino-polycarboxylic acids a shift of the CO_2^- antisymmetric frequency on addition of one proton is also observed. Since in all cases only one band is found, at ~1630 cm.$^{-1}$, it is deduced that all the available carboxylate groups interact in an equivalent manner with the single proton. Thus the monoprotonated form of methyliminodiacetic acid, is represented in (II). Molecular models show that such structures are possible sterically: two or three rings of type (I) may be built up around one nitrogen atom with out steric hindrance.

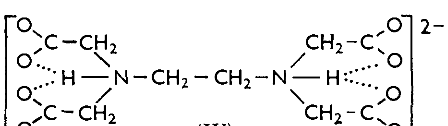
Glycine has been shown by neutron diffraction to contain a bifurcated hydrogen bond,[22] and others have been suggested;[16] that in glycylglycine[22] is less well established.

Methyliminodiacetic Acid, MIDA.—In solution the first proton is added to the nitrogen atom. The infrared spectra of the methyliminodiacetate ion, Z^{2-} in water and heavy water solution show a band due to the C–N vibration at 1120 cm.$^{-1}$, whilst the singly charged ion HZ^- does not. The n.m.r. results confirms this. The CH_3 and CH_2 groups have similar $\Delta\tau$ values for addition of the first proton (0·67, 0·70 p.p.m.; cf. Table 2, data for dimethylglycine), confirming the view that this proton is added to the nitrogen atom. The $\Delta\tau$ values for CH_2 for addition of the second and the third proton are almost identical (0·20, 0·27 p.p.m.) and are much smaller: their sum (0·47 p.p.m.) is similar to that for the second proton addition to dimethylglycine (0·39 p.p.m.). The effect of adding the second proton to methyliminodiacetic acid is averaged between the two carboxylate groups. The small changes in τ for the CH_2 group in the last two proton additions (0·10, 0·10 p.p.m.) may be due to breaking of the hydrogen bonds.

Examination of the infrared spectra in heavy water solution indicates the presence of hydrogen-bonded units: the CO_2^- (as) frequency of the methyliminodiacetate ion, Z^{2-}, is raised by 42 cm.$^{-1}$ on protonation, and since only one peak is observed in this region the corresponding hydrogen anion, HZ^-, probably exists in solution with structure (II). Addition of a further proton produces bands at 1730 and 1631 cm.$^{-1}$; the higher-frequency band indicates a CO_2H group: the lower-frequency one is still much higher than corresponds with the free carboxylate in Z^{2-}. A hydrogen-bonded ring of type (I) probably occurs, so that the structure of methyliminodiacetic acid is (III).



(III)



(IV)

Addition of a further proton breaks the ring, giving two equivalent CO_2H groups with frequency 1734 cm.$^{-1}$.

The values of Δ in Table 2 confirm these results: the anion, Z^{2-}, has Δ 176 cm.$^{-1}$; the hydrogen anion, HZ^-, and the acid, H_2Z, have Δ 222 and 223 cm.$^{-1}$, respectively, which indicates that in the hydrogen anion and the acid there is strong hydrogen bonding.

The solid state spectrum of methyliminodiacetic acid has no frequencies which may be associated with a carboxylate group, so that, in contrast with its state in the solution, the solid acid is in a normal form.

Ethylenediaminetetra-acetic Acid, EDTA.—This acid might be expected to behave as

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[22] Fuller, *J. Phys. Chem.*, 1959, **63**, 1705.

two methyliminodiacetic acid molecules joined together, and this has been largely confirmed. The intermediate forms, H_3Y^- and H_5Y^+, do not exist independently in solution because the p*K* value of H_3Y^- is close to that of H_4Y; a similar argument applies to H_5Y^+ and H_6Y^{2+}. However, the monoprotonated form, HY^{3-}, can be obtained essentially pure at least in solution, because it has an intermediate p*K* value (see footnote, p. 3650). This form is discussed below.

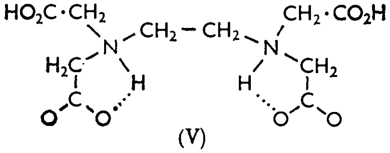
Addition of a further proton produces the characteristic single band at 1628 cm.$^{-1}$; the carboxylate frequencies have all been raised by 43 cm.$^{-1}$. Probably the ion H_2Y^{2-} exists as a double zwitter-ion containing two double rings with each CO_2^- group hydrogen bonded to NH^+ (IV).

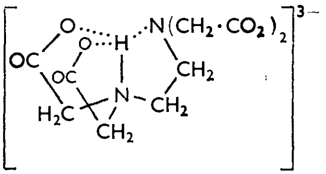
Addition of two further protons should, by analogy with methyliminodiacetic acid, produce two CO_2H groups and two CO_2^- groups hydrogen-bonded to NH^+. The spectrum * of the acid D_4Y in heavy water shows bands at 1725 and 1626 cm.$^{-1}$ which correspond to this structure (V).

Further addition of two protons produces four identical CO_2H groups with a frequency of 1715 cm.$^{-1}$.

The water-solution spectra in the 1100 cm.$^{-1}$ region show that on the addition of two protons to the ethylenediaminetetra-acetate ion, Y^{4-}, the band assigned to C–N disappears. This confirms the double zwitter-ion structure (IV).

The infrared spectrum of trisodium deuterium ethylenediaminetetra-acetate, Na_3DY, in heavy water shows two peaks of similar intensity at 1625 and 1585 cm.$^{-1}$. Two carboxylate groups must be hydrogen-bonded to NH^+, and two are " free." This is confirmed by plotting extinction coefficients at 1625 and 1585 cm.$^{-1}$ for a series of Na_4Y–Na_3DY–Na_2D_2Y mixtures against the average number of protons per ethylenediaminetetra-acetate ion. Straight lines were obtained: if, in the anion, HY^{3-}, any number of CO_2^- groups other than two were involved in hydrogen-bonding, a change in slope would be observed at the composition of the trisodium salt. The infrared spectrum of trisodium ethylenediaminetetra-acetate in ordinary water shows a strong peak at 1115 cm.$^{-1}$, which indicates the presence of one nitrogen atom not strongly bound to H^+.


(V)


(VI)

However, the structure of the monoprotonated anion in solution cannot be simply one double ring of type (II) and one completely free nitrogen atom, since this does not explain the intermediate p*K* value for $Na_2H_2Y \rightleftharpoons Na_2HY + H^+$. Schwarzenbach and Ackermann [1] suggest, in order to explain this, that the single proton is hydrogen-bonded between the two nitrogen atoms. Combining this with the above infrared evidence, the structure shows an interaction stereochemically equivalent to a trifurcated hydrogen bond † as in (VI). The structure is analogous to that suggested for the monoprotonated form of the nitrilotriacetate anion.

The n.m.r. evidence is in good agreement with the structures deduced above except for

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* The CO_2H bands are observed to be less intense than the carboxylate bands. Although ethylenediaminetetra-acetic acid, H_4Y, methyliminodiacetic acid, H_2Z, and nitrilotriacetic acid, H_3X, exist in solution as zwitter-ions, the CO_2H peak height of all of them is less than one-third that of the CO_2^- peak. Ethylenediaminetetra-acetic acid and nitrilotriacetic acid, which are only slightly soluble, were examined in heavy water and in 0·01M- and 0·05M-deuterium chloride, to ensure that the effect is not due to ionisation in heavy water. The relative intensities of the peaks remained constant.

† Kinetic data on the oxidation of EDTA with manganic salts supports the view that, whilst the monoprotonated anion, HY^{3-}, has free carboxylate groups, the diprotonated anion, H_2Y^{2-}, has none.[23]

[23] Hamm, personal communication.

the ethylenediaminetetra-acetic acid spectrum in supersaturated solution (see below). The $\Delta\tau$ values for the " inner " and " outer " CH_2 groups are both large for addition of the first two protons and much smaller for that of the next four. This confirms the addition of the first two protons to nitrogen. The magnitude of the shift for the inner CH_2 protons is larger than that for the outer ones (inner $\Delta\tau$ 1·20, outer $\Delta\tau$ 0·85 p.p.m.); the " outer " CH_2 groups are attached only to one NH^+ group, whereas the inner CH_2's are between two NH^+ groups. This effect is also observed in the model compound tetramethylethylenediamine, where $\Delta\tau$ for the CH_3 groups (0·51 p.p.m.) is less than that for the CH_2 groups (0·77 p.p.m.) by 0·26 p.p.m. whereas in *NN*-dimethylglycine and *N*-methyliminodiacetic acid the difference of $\Delta\tau$ for CH_3 and CH_2 is only 0·13 p.p.m. The value of $\Delta\tau$ for the outer CH_2's on changing from H_2Y^{2-} to H_6Y^{2+} (0·55 p.p.m.) is of the same order as that for the CH_2 in the second protonation of *NN*-dimethylglycine (0·39 p.p.m.); this is further evidence that this stage is one in which CO_2^- groups are protonated. There is also a considerable $\Delta\tau$ value for the inner CH_2 protons (0·34 p.p.m.) in this stage. This is ascribed to the breaking of the hydrogen bonds, making the NH^+ groups more positive (cf. *N*-methyliminodiacetic acid). The effect is accentuated because the CH_2's are between two such NH^+ groups.

The spectrum of ethylenediaminetetra-acetic acid, H_4Y, is expected to show $\Delta\tau$ values intermediate between those of the dihydrochloride, H_6Y^{2+}, and diprotonated anion, H_2Y^{2-}. However, the solubility of the acid in water is too low for the compound to give an observable signal, so spectra of supersaturated solutions were taken. At a concentration of ethylenediaminetetra-acetic acid in water large enough to give a detectable signal in 5-mm. tubes (0·25M, equivalent to about 100-fold supersaturation), fairly consistent spectra were obtained giving averages of 6·53 (inner CH_2) and 6·00 p.p.m. (outer CH_2). These values diverge from the expected pattern. The value for the inner CH_2 has increased from that for the diprotonated anion, H_2Y^{2-} (6·35 p.p.m.), whereas it would be expected to decrease. The value is, however, consistent with that expected for the normal form of the acid: the increase of $\Delta\tau$ for the inner CH_2 protons is then due to removal of protons from the nitrogen atoms to the CO_2^- groups, giving a structure containing four carboxylic acid groups.

At lower concentrations, which required the use of a wider tube with consequent loss of resolution, only one rather broad peak was observed. The spectrum of the dideuterated anion (0·025M-D_2Y^{2-}) in heavy water was measured under those conditions, and a peak at τ 6·15 p.p.m. was found, which corresponds to an average, weighted according to the intensities, of the two peaks found for the diprotonated anion, H_2Y^{2-}, in water under the higher resolution. On acidification, to produce a solution 0·025M in ethylenediaminetetra-acetic [$^2H_4$]acid, D_4Y, the peak shifts to τ 6·08 p.p.m. This does not correspond to a weighted average (6·18 p.p.m.) of the two peaks found in 0·25M-ethylenediaminetetra-acetic acid, H_4Y: it is slightly nearer the expected average (~6·00 p.p.m.) for the acid in a zwitter-ion form.

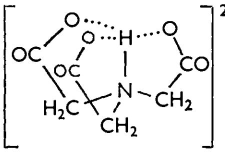
We consider that at high supersaturation the spectrum is probably concentration-dependent and at lower concentrations approaches that expected for a double zwitter-ion. A possible explanation of these anomalous results is that, before crystallising, the zwitter-ion form rearranges in solution to give the normal form found in the crystal (see below).

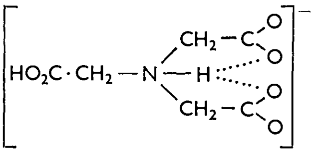
The weight of evidence is in favour of ethylenediaminetetra-acetic acid's existing in solution and ionising as a double zwitter-ion. The solid-state infrared spectrum of the acid is very different from that in solution: a single band at 1695 cm.$^{-1}$ interpreted as showing that the acid is in a " normal " form. The change of structure on crystallisation appears to be the principal reason for the disparity between the two investigations previously reported.[2,3]

A small difference between the infrared spectra of solid and solution is observed for the disodium salt, Na_2H_2Y: the crystals have a prominent shoulder (1658 cm.$^{-1}$) at the side of the CO_2^- (as) frequency (1628 cm.$^{-1}$). This may be due to a strongly hydrogen-bonded

CO_2H:[2] the frequency lowering of such a band (78 cm.$^{-1}$) is so large that there is no sharp distinction between CO_2H and CO_2^-. The shoulder indicates that the hydrogen bonding in the crystal is less symmetric than in solution: whether it is inter- or intra-molecular is not easily determined.

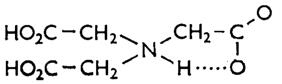
Nitrilotriacetic Acid, NTA.—The infrared spectra, in heavy water solution, of the mono-deuterated anion, DX^{2-}, and the bare anion, X^{3-}, indicate that one proton is sufficient to raise all the CO_2^- frequencies by 37 cm.$^{-1}$. The spectra of water solutions are consistent with the pK measurements and show that the first proton is added to nitrogen. The anion, HX^{2-}, must therefore have all three CO_2^- groups equally bonded to the NH^+ in an interaction stereochemically equivalent to a " trifurcated " hydrogen bond (VII). A model of this structure showed that there is no interference between the three rings. Such a hydrogen bond has not previously been reported.

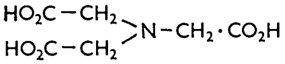

(VII)


(VIII)

Addition of a further proton gives the two-ring structure, with one CO_2H group (VIII), deduced from the observed bands at 1635 and 1730 cm.$^{-1}$.

The next proton merely intensifies the 1730 cm.$^{-1}$ band and lowers the intensity of the 1635 cm.$^{-1}$ band; however, the latter band is still the stronger (see footnote, p. 3654). It has been suggested,[24] on the basis of pK measurements, that nitrilotriacetic acid, H_3X, is not a zwitter-ion in solution although more accurate measurements [25] extrapolated to infinite dilution show that this suggestion is unnecessary. The infrared solution spectrum, cannot be reconciled with a " normal " form of nitrilotriacetic acid. The structure deduced from the spectrum is (IX). Further protonation, to the hydrochloride containing the ion, H_4X^+, produces three identical CO_2H groups with frequency 1732 cm.$^{-1}$. As with *N*-methyliminodiacetic acid and ethylenediaminetetra-acetic acid, the spectra indicate that nitrilotriacetic acid, though a zwitter-ion in solution, takes up a " normal " form (X) in the solid.


(IX)


(X)

The infrared spectrum of the crystalline disodium salt, Na_2HX, shows two bands of similar intensity in the CO_2H–CO_2^- region, at 1673 and 1632 cm.$^{-1}$. All the carboxylate groups must be strongly hydrogen-bonded: the two peaks may be due to different site symmetry within the crystal, or to CO_2^- groups hydrogen-bonded to different extents. Since there is only one proton for three carboxylate groups, the structure may involve interactions equivalent to bifurcated and trifurcated hydrogen bonds.

The Δ value for the " more strongly bonded CO_2^- " (312 cm.$^{-1}$) is the highest found in this investigation.*

The n.m.r. results, with one exception, are consistent with this. For the first three protons, values of both $\Delta\tau$ (0·67, 0·21, 0·19 p.p.m.) and τ are very similar to those for the

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* It is probable, following previous arguments, that the higher-frequency CO_2^- (as) vibration and the lower-frequency CO_2^- (sym) vibration are associated with the same carboxylate group. If this were not so, both oxygen atoms of each CO_2^- group would be involved in H bonding.[13a]

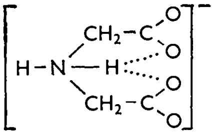
[24] Schwarzenbach, Kampitsch, and Steiner, *Helv. Chim. Acta*, 1945, **28**, 828.
[25] Hughes and Martell, *J. Amer. Chem. Soc.*, 1956, **78**, 1319.

CH_3 protons of *N*-methyliminodiacetic acid, so the first proton is added to the nitrogen atom, and subsequent ones to the CO_2^- groups. There is no support for the suggestion [24] that nitrilotriacetic acid is not a zwitter-ion in solution. The spectrum of the acid was taken under conditions of very high supersaturation, conditions under which ethylenediaminetetra-acetic acid does take up a normal form.

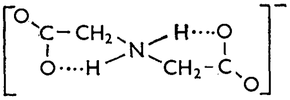
The puzzling feature of this set of spectra is the very large $\Delta\tau$ for the last proton addition (0·99 p.p.m.).

Iminodiacetic Acid, IDA.—Comparison of the infrared spectra of the forms of iminodiacetic acid with the corresponding forms of *N*-methyliminodiacetic acid suggests that the two have the same ionisation pattern, and corresponding forms have corresponding structures.

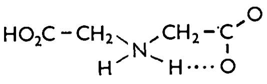
However, the monoprotonated anion, HW^-, may have structure (XI) or (XII). Both are compatible with the infrared spectrum, which shows only a single frequency at 1618 cm.$^{-1}$. Addition of another proton produces a species (XIII) with bands at 1727 and 1624 cm.$^{-1}$. Addition of the last proton produces two identical CO_2H groups with frequency of 1728 cm.$^{-1}$. The ambiguity of the nature of the monoprotonated anion is resolved with the aid of the n.m.r. data.



(XI)



(XII)



(XIII)

If the ring structure were that of (XI), the ionisation pattern would be the same as for *N*-methyliminodiacetic acid and the changes in the τ value for the CH_2 protons should exactly parallel those of that acid. The observed changes on stepwise protonation are 0·70, 0·25, 0·27 p.p.m. for the latter and 0·19, 1·32, 0·24 p.p.m. for iminodiacetic acid. As expected, the values for the last stage, in which infrared data indicate similar changes for the two substances, are very similar. However, for the first two proton additions, the relative magnitudes of $\Delta\tau$ are reversed.

We consider that the values of $\Delta\tau$ for iminodiacetic acid indicate the double-ring structure (XII) for the monoprotonated anion, HW^-. In this structure, the positive charge, which normally, when brought up to the nitrogen atom, exercises a strong attraction upon the screening electrons of the neighbouring CH_2 protons, is shared between two (N–H) protons, and the attraction is greatly reduced. This sharing effect may be compared with the exchange effect noted above for the addition of a proton to one of several carboxylate groups. In the one case the effect is brought about by an equal sharing, in time, of a whole charge at different positions; in the other, by an equal sharing in space of two half-charges. Since the n.m.r. spectrum cannot observe position differences if they alternate in less than a certain time ($\sim 10^{-2}$ sec.), it will not distinguish between averaging effects due to position-sharing and to time-sharing. The values of $\Delta\tau$ are similar for the proton addition to a CO_2^- of *N*-methyliminodiacetic acid (0·25 p.p.m.), and for addition of this first proton to iminodiacetic acid (0·19 p.p.m.).

Addition of the next proton breaks this two-ring structure, unique among the compounds examined, forming a single-ring structure of type (II), which occurs at some point in the ionisation pattern of all the compounds $NRR'\cdot CH_2\cdot CO_2H$. In all the α-aminopolycarboxylic acids there is a considerable $\Delta\tau$ for the addition of sufficient protons to produce the single-ring form of *N*-methyliminodiacetic acid ($\Delta\tau$ 0·95 p.p.m.). It is therefore to be expected that iminodiacetic acid will also show a large $\Delta\tau$ for addition of two protons. Since the first proton produced a small $\Delta\tau$, the second should produce a large $\Delta\tau$. The observed large value for addition of the second proton probably arises from the asymmetry of the hydrogen atoms attached to nitrogen in iminodiacetic acid: the positive charge shared between the two hydrogen atoms in the monoprotonated anion, HW^-, is now localised largely upon the hydrogen atom involved in the ring hydrogen bond.

Conclusion.—We conclude that all the amino-acids examined form internally hydrogen-bonded ions, and that the polycarboxylic acids are capable of displaying interactions stereochemically indistinguishable from internal, branched, or bifurcated hydrogen bonds in aqueous solution. All the amino-acids examined behave as zwitter-ions in solution, including those whose nature had been previously questioned. The first cases of structure change, from zwitter-ion to a " normal " form, on crystallising from solution are reported.

The deductions rest primarily upon infrared spectra and it is clear that the examination of both heavy water and water solutions is an experimentally simple but powerful means of structure determination. The examination of n.m.r. spectra of solutions of a series of salts of an acid is a useful additional method of examining the ionisation pattern; however, as is shown by the data for nitrilotriacetic acid, the method could be misleading if used alone on a single acid. When more than one form is present in solution, the more exact, but laborious, technique recently reported by Loewenstein and Roberts[17] for citric acid would have to be used, though even here there are difficulties of interpretation.[26]

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[26] Martin, *J. Phys. Chem.*, 1961, **65**, 2053.