760. *The Rotatory Dispersion of* L(+)-α-*Alanine.*

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The rotatory dispersion of L(+)-α-alanine in various solvents at several temperatures has been examined in the visible region.

Sørensen [1] showed that amino-acids could be titrated like other acids in the presence of formaldehyde, assuming that the formaldehyde blocked the amino-group, probably by the formation of a compound of Schiff's base type. But Levy [2] considered the equilibria governing this titration to be:

$$^{+}NH_3\cdot CHR\cdot CO_2^{-} \rightleftharpoons NH_2\cdot CHR\cdot CO_2^{-} + H^{+}$$

$$NH_2\cdot CHR\cdot CO_2^{-} + CH_2O \rightleftharpoons (CH_2\cdot O\cdot NH_2)\cdot CHR\cdot CO_2^{-}$$

$$NH_2\cdot CHR\cdot CO_2^{-} + 2CH_2O \rightleftharpoons [(CH_2O)_2NH_2]\cdot CHR\cdot CO_2^{-}$$

Thus the reaction between formaldehyde and the amino-acid should occur only when the latter is in its " neutralised " or deprotonated form. Svehla [3] has shown, however, by molecular-weight measurements, that weakly bonded compounds are formed between formaldehyde and amino-acids even in simple aqueous solutions. Levy considered these to be of no importance in the Sørensen titration, but Loiseleur and Crovisier [4] continued to

[1] Sørensen, *Biochem. Z.*, 1908, **7**, 45.
[2] Levy, *J. Biol. Chem.*, 1932—33, **99**, 767.
[3] Svehla, *Ber.*, 1923, **56**, 331.
[4] Loiseleur and Crovisier, *Bull. Soc. Chim. biol.*, 1942, **24**, 241.

accept the Sørensen explanation, assuming that a large change in pH occurs when formaldehyde is added to an aqueous solution of the acid. This assumption is hardly supported by results obtained in the present work, which was carried out at similar concentrations to those of Loiseleur and Crovisier, the observed pH change being 0·2 unit and thus insufficient to explain the altered titration behaviour.

Loiseleur and Crovisier also showed that amino-acids may undergo mutarotation at pH 7 in the presence of formaldehyde. Frieden, Dunn, and Coryell [5] observed an exaltation of the rotatory power of amino-acids (in the anionic form) in the presence of formaldehyde, but in this case no mutarotation was found. Mutarotation of ethanolic solutions of amino-esters on addition of acetone has been recorded by Bergel and Lewis,[6] who considered that this might be due to the formation of an amino-alcohol by interaction of the amino-group and the keto-group of the acetone:

$$EtO_2C{\cdot}CHR{\cdot}NH_2 + OCMe_2 \rightleftharpoons EtO_2C{\cdot}CHR{\cdot}NH{\cdot}CMe_2{\cdot}OH$$

Since they recovered some of their starting material (when this was crystalline, *e.g.*, tyrosine ethyl ester) merely by precipitation with a hydrocarbon or, more effectively, by evaporation in the cold, it seems that any bonds formed are of low energy. Professor Bergel (in a personal communication) has stated that there is evidence that in non-aqueous solvents the reversible equilibrium is carried a stage further, to the formation of the Schiff's base with elimination of water.

Such systems lend themselves to study by means of their optical rotatory dispersion. As a preliminary, one of Bergel and Lewis's amino-acids, (+)-α-alanine, has been examined. Cursory studies have previously been made by Patterson and Brode [7] and by Brand *et al.*[8] The low solubility of alanine even in solutions containing only 10% of organic solvent severely restricted the scope of our work: as far as possible the measurements were carried out at a fixed alanine concentration of 10%. More dilute solutions are preferable but even at this concentration the observed rotations for green light are only of the order of half a degree.

Results.—When acetone, dioxan, or mono- or di-methylformamide is added to an aqueous solution of alanine there is a general exaltation of the rotatory power in the visible region. On the other hand, increasing the temperature of the first three of these solutions reduces their rotatory power, as is the case with the solution in pure water Within the limits of experimental error the reductions in rotatory power are identical (Table 1).

TABLE 1. *Effect of temperature on the rotatory power * of* L(+)-α-*alanine in various solvents.*

Solvent	*c*	Molar ratio, active agent : alanine	$[M]_{5461}^{25\cdot0}$	$[M]_{5461}^{41\cdot1}$	Diff.
4·5% Aq. NHMe·CHO	10	0·7 : 1	3·10°	2·38°	0·72°
Aq.	10	—	2·60	1·98	0·62
3% Aq. $COMe_2$	10	0·5 : 1	2·73	2·00	0·73
10% Aq. $COMe_2$	10	1·5 : 1	3·21	2·35	0·66
11% Aq. dioxan	10	1·2 : 1	3·90	3·19	0·71
2·5N-HCl	3·4	6·6 : 1	15·13	14·05	1·08
0·9N-HCl	4	2·3 : 1	15·22	14·10	1·12
Water	5	—	1·87	1·25	0·62
1·08N-NaOH	4·8	2 : 1	5·06	3·64	1·42
1·62N-NaOH	4·8	3 : 1	5·27	3·50	1·77

* The figures for [*M*] are interpolated from Tables 2 and 3.

The effect of temperature on the acid, " neutral," and alkaline solutions can be compared in the same way. In this case the largest reductions are observed with the

[5] Frieden, Dunn, and Coryell, *J. Phys. Chem.*, 1943, **47**, 10, 20.
[6] Bergel and Lewis, *Chem. and Ind.*, 1955, 774.
[7] Patterson and Brode, *Arch. Biochem.*, 1943, **2**, 247.
[8] Brand, Washburn, Erlanger, Ellenbogen, Daniel, Lippman, and Scheu, *J. Amer. Chem. Soc.*, 1954, **6**, 5037.

alkaline solutions; the acid solutions show a rather smaller effect but with the aqueous solutions the reduction is about half that observed for the other two. A full analysis of the temperature effect by the equilibrium method [9] would be desirable but the relative inaccuracy of these measurements renders success in this unlikely. The strong dependence of the rotation on temperature suggests that the rotatory power is very dependent on an equilibrium of different forms, particularly in alkaline and acid solution. The actual variation of the rotatory power of the aqueous solutions is rather similar to that observed for octan-2-ol,[10] though the percentage variation is much higher. The production of negative rotations at the higher temperatures shows that at least one of the equilibrium forms has a negative rotatory power (see Table 2).

TABLE 2. *Variation in the molecular rotatory power of* L(+)-α-*alanine with concentration in aqueous solution and with hydrogen-ion concentration* (l = 2 dm.).

			Wavelength (Å)							$[M]_{4358}$
Solvent	c *	Temp.	6438	5893	5780	5461	5086	4800	4358	$[M]_{5461}$
H_2O	10	24·8°	1·33°	2·21°	2·30°	2·60°	3·41°	3·78°	5·46°	2·10
	10	35·4	1·18	—	1·92	2·22	2·99	3·38	4·77	2·15
	10	45·4	0·90	—	1·50	1·72	2·39	2·79	3·83	2·23
	10	53·8	0·53	—	1·16	1·35	1·80	2·21	2·92	2·16
H_2O	7·5	25·3	1·24	—	2·05	2·31	2·97	3·45	5·05	2·18
	7·5	55·3	0·83	—	0·78	0·95	1·16	1·56	2·31	2·43
	7·5	67·1	0·90	—	0·44	0·56	0·61	0·86	1·45	2·58
	7·5	73·3	0·32	—	0·06	0·15	0·09	0·39	0·63	4·2
	7·5	95·3	—	—	−0·98	−1·06	—	—	−1·31	1·23
H_2O	5	25·0	0·59	—	1·61	1·87	2·44	2·80	3·40	1·82
	5	38·0	0·14	—	1·14	1·38	1·79	2·21	2·84	2·05
	5	56·0	−0·22	—	0·46	0·66	1·04	1·00	1·37	2·07
	5	69·0	−0·17	—	—	−0·07	−0·07	—	—	—
0·9N-HCl	4	25·2	9·86	12·51	13·23	15·22	18·00	21·03	28·36	1·86
	4	45·0	8·49	—	12·36	13·92	17·13	—	26·49	1·90
	4	66·9	8·03	—	11·28	13·03	16·41	—	24·56	1·88
	4	96·7	6·78	—	9·98	11·49	14·20	—	21·72	1·89
2·5N-HCl	3·4	25·2	8·96	—	13·16	15·13	18·29	20·70	26·88	1·77
	3·4	47·4	7·63	—	11·84	13·57	16·15	—	24·92	1·96
	3·4	64·6	6·87	—	10·92	12·70	15·35	—	23·41	1·84
	3·4	97·1	5·96	—	9·93	11·33	13·38	—	20·69	1·82
1·08N-NaOH	4·8	25·3	3·41	4·10	4·47	5·06	6·02	6·16	8·45	1·67
	4·8	47·6	1·62	—	2·81	3·08	3·68	3·53	5·15	1·67
1·62N-NaOH	4·8	25·3	2·59	3·95	4·51	5·27	6·51	6·99	8·10	1·54
	4·8	48·0	0·74	—	2·49	2·77	3·81	3·83	3·91	1·41
	4·8	93·2	−1·05	—	−1·04	−1·15	−1·71	—	2·16	1·87

* The "c" values at the higher temperatures are approximate; the values used were corrected for the expansion of the solution.

Addition of acetone to acid solution has virtually no effect on the rotatory power, but dioxan exalts it (Table 3), as in the "neutral" solution. On the other hand, addition of dioxan to an alkaline solution of alanine, if anything, reduces the rotatory power slightly, whereas addition of acetone reduces it most markedly, to a value similar to that observed in pure water.

Discussion.—The effect of acetone on the aqueous solution is in a similar sense to that observed by Bergel and Lewis [6] for an ethanolic solution of the ester, though it is less pronounced. (see Table 4). Owing to the small value of the rotatory power it is hardly feasible to follow any rapid change kinetically, but no detectable mutarotation over a long period was noticed. The distinction drawn by Levy between the action of formaldehyde on amino-acids in the presence and absence of an equivalent amount of alkali is supported by the rotatory behaviour of alanine in aqueous acetone in the presence of acid and of alkali (Tables 3 and 4).

[9] Hargreaves, *J.*, 1957, 1071.
[10] Hargreaves, *J.*, 1953, 2953.

TABLE 3. *Effect of organic solvents on the molecular rotatory powers of aqueous acid and alkaline solutions of* L(+)-α-*alanine.*

Solvent	*c*	Temp.	$[M]_{5461}$
1·62N-NaOH	4·8	—	5·27
1·49N-NaOH + acetone (6·4%w/v)	4·4	25·2°	2·30
	4·4	38·7	2·14
1·49N-NaOH + dioxan (9·5%)	4·4	25·2	5·12
2·5N-HCl	3·4	25·2	15·09
2·26N-HCl + acetone (9·0%)	3·1	25·2	15·08
2·26N-HCl + dioxan (12·0%)	3·1	25·2	16·79

TABLE 4. *Molecular rotatory powers of* L(+)-α-*alanine in various aqueous solvents* ($l = 2$ dm.).

			Wavelength (Å)							$[M]_{4358}$
Solvent	*c* *	Temp.	6438	5893	5780	5461	5086	4800	4358	$[M]_{5461}$
3% Aq. $COMe_2$	10	25·2°	1·39°	—	2·38°	2·73°	3·31°	3·86°	5·57°	2·04
	10	41·1	1·05	—	1·68	2·00	2·47	2·92	4·07	2·03
10% Aq. $COMe_2$	10	25·4	1·98	2·54°	2·65	3·17	4·03	4·46	5·78	1·82
	10	39·8	1·25	—	2·09	2·38	2·95	3·43	4·50	1·89
	10	51·6	1·01	—	1·63	1·85	2·34	2·67	3·30	1·78
11% Aq. Dioxan	10	25·2	2·46	3·13	3·47	3·88	4·78	5·56	7·39	1·90
	10	42·2	1·69	—	2·75	3·12	3·79	4·36	6·30	2·01
	10	60·7	1·33	—	1·99	2·31	2·66	3·04	4·48	1·94
	10	75·6	0·76	—	1·34	1·51	1·63	—	3·19	2·11
	10	96·4	—	—	0·45	0·59	—	—	1·16	1·97
4·5% Aq. NHMe·CHO	10	25·0	2·47	2·61	2·68	3·10	3·85	4·23	6·20	2·00
	10	39·1	1·74	—	2·19	2·44	3·13	—	5·29	2·16
	10	64·4	0·73	—	1·11	1·28	1·72	—	2·88	2·25
	10	94·9	—	—	−0·31	−0·30	—	—	−0·22	—
22% Aq. NHMe·CHO	6·7	25·3	2·67	3·46	3·68	4·27	5·12	—	8·27	1·93
	6·7	40·4	1·87	—	2·91	3·35	4·00	—	6·49	1·94
	6·7	60·9	1·30	—	1·97	2·29	2·79	—	4·26	1·86
	10	95·0			0·45	0·55	—	—	1·90	3·45
Aq. NMe_2·CHO	10	25·3	2·89	3·19	3·43	3·98	5·20	5·91	7·75	1·94

* Cf. Table 2.

The rotatory behaviour of α-alanine in neutral aqueous acetone may be ascribed to compounds similar to those shown by Svehla [3] to exist between amino-acids and formaldehyde. Formation of these compounds may merely give preference to a particular conformation of the optically active molecule or it may act more directly by influencing the asymmetry of the electronic field. Bergel and Lewis's results, in particular, indicate that the compounds formed under these conditions are unstable. The exaltation which occurs in mono- and di-methylformamide might also be attributed to similar compound formation between the amino-acid and the keto-carbonyl group of these molecules.

The effect of adding dioxan to the aqueous solution cannot, however, be ascribed to a similar cause, and its effect is noticeably greater than that of acetone.

The exaltation produced by dioxan might be explained in terms of the increase of the dissociation constant of the amino-acid with reduction of the dielectric constant of the solvent, particularly since alanine in sodium hydroxide solution shows a rather greater exaltation. This is, however, contrary to the available evidence which shows that dissociation is suppressed by lowering the dielectric constant of the medium. Quantitatively this behaviour can be explained in terms of variation in the dissociation of the acid only if its anion can be presumed to have a large negative rotatory power. A plot of the available figures for the variation of the dissociation constant of lysine [11] with the dielectric constant of its aqueous-ethanolic solvent (for this purpose the dielectric constants are assumed to be approximately additive) shows that for a 10% solution of dioxan in water

[11] Schmidt, " The Chemistry of the Amino Acids and Proteins," Thomas, Springfield, 1938, p. 195; Jukes and Schmidt, *J. Biol. Chem.*, 1934, **105**, 359.

(ε 72) the pK_a should be 2·32, compared with 2·18 in water. Although the actual figures for alanine would be different their variation may be expected to be similar. This variation corresponds to a change in the concentration in the alanine anion of 1 part per 100 parts of alanine. The observed exaltation (*ca.* 1·3°) in the dioxan solution corresponds, therefore, to a value of about −150° for the molecular rotation of the alanine anion at 5461 Å. A direct check on this figure is not possible as there is evidence [12] that even alkali salts of weak organic acids are engaged in complex-formation, but in view of the observed rotation of the acid in sodium hydroxide solution (*ca.* +5°) the value of −150° seems very unlikely.

Also against a simple interpretation in terms of the variation of the dielectric constant of the solvent are the rotations obtained in the presence of substituted formamides. Both these added solvents raise the rotatory power of an aqueous solution of alanine. Dimethylformamide (ε 38·6) raises the rotatory power rather more than does the monomethyl compound (ε 190·5),[13] but both exalt the rotatory power though one may be expected to reduce the dielectric constant of the medium and the other to raise it. In this case it is possible that compound formation and the electrical effect may be both active, either reinforcing or opposing each other. From this also it appears that the dielectric effect must be too small to make the major contribution to the change.

In considering the effect of the solvents their electrical nature must, of course, be taken into account. Lutz and Jirgensons [14] have shown, for example, that for alanine [*M*] has an almost constant value with the variation of the acid concentration above a molar acid : amino-acid ratio of 6 : 1. Below this figure the rotation drops sharply to the neutral value, and then rises slowly with the alkali concentration. Thus the cation $^{+}NH_3{\cdot}CHR{\cdot}CO_2H$ apparently has a much higher rotation than the zwitterion, $^{+}NH_3{\cdot}CHR{\cdot}CO_2^{-}$, or the anion, $NH_2{\cdot}CHR{\cdot}CO_2^{-}$. Table 3 shows that the addition of acetone to the acid solution of alanine has no detectable effect on the rotatory power. At these acid strengths Lutz and Jirgensons found no large variation of rotatory power with concentration. On the other hand, dioxan raises the rotatory power by approximately the same amount as in neutral solution. The position is reversed in the alkaline solutions; in this case the addition of dioxan has virtually no effect on the rotatory power, whilst the addition of acetone reduces the rotatory power of the solution to half its value, so that it approximates to that in neutral solution. Thus it seems probable that if the acetone exerts its influence through the amino-group the effect of the dioxan is exerted through the carboxyl group. In addition it is noteworthy that the effect of temperature on both the cationic and the anionic form is apparently greater than on the neutal form. The elucidation of this behaviour clearly involves a discussion of the structure of the zwitterion; it is hoped to undertake this later.

Experimental

L-Alanine (from Roche Products) gave only a single spot when its paper chromatogram was run with either the butanol–acetic or the phenol–water solvent and developed with ninhydrin.

The rotatory powers were correct to ±0·005° for 5780 and 5461 Å, and about ±0·03° for the other lines. Temperatures were constant within ±0·2°, and were measured in the solution.

Solvents were distilled immediately before use.

To 0·0509N-alanine solution (50 c.c.) of pH 5·91 was added formaldehyde solution (23·1% of formaldehyde, as determined by alkaline iodine) (5 c.c.) of pH 6·14, obtained by distilling " 40% formalin " from sodium carbonate and collecting the distillate in water; the pH after mixing reached a steady value of 5·70.

With solutions more concentrated in formaldehyde than those used by Loiseleur and Crovisier [4] the change is rather greater than this, *viz.*: 25 c.c. of the above alanine solution

[12] Hargreaves and Richardson, *J.*, 1957, 2260.
[13] Leader and Gormley, *J. Amer. Chem. Soc.*, 1951, **73**, 5731.
[14] Lutz and Jirgensons, *Ber.*, 1930, **63**, 448.

(pH 5·91), mixed with 25 c.c. of the formaldehyde solution of pH 6·14, gave a solution with pH 5·27.

The change in pH of formaldehyde solution on dilution with water corresponds to additivity of the hydrogen-ion concentrations: 5 c.c. of the above formaldehyde solution (pH 6·33), diluted with 50 c.c. of water (pH 6·00), gave a solution of pH 6·06.

No significant change was observed on dilution of alanine: 0·0509N-alanine (pH 6·13), diluted with an equal volume of water (pH 6·08), gave a solution with pH 6·12.

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