Enhancement of the Optical Rotation of α-Amino-acids by Formation of Carbamate (*N*-Carboxy-) Salts

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The optical rotations of fourteen L-α-amino acids dissolved in aqueous potassium carbonate became more positive with time, eventually reaching limiting values, with optical activity enhancements ranging from <1 to >50 fold. The ^{1}H n.m.r. signals from the α-protons showed a concomitant down-field shift. These changes are explained by the formation of carbamate (*N*-carboxy-) salts. The ^{13}C n.m.r. spectra also altered with time, showing the gradual appearance of a signal assigned to the carbamate carbon atom.

In an attempt to measure the racemization of L-serine in aqueous potassium carbonate (pH 10·2) we found that the (negative) optical rotation decreased rapidly to zero and then increased again, but in the positive direction. Equilibrium was attained overnight. Sodium hydroxide solution containing L-serine at the same pH value, on the other hand, revealed no change in optical rotation during 24 h. D-Serine behaved analogously, acquiring a negative rotation in aqueous potassium carbonate. The behaviour of fourteen optically active α-amino-acids was then studied; all showed optical activity enhancements which ranged from <1 to >50 fold. The rate of change of optical rotation varied according to the amino-acid; approximate half-lives are given in Table 1.

These changes can be explained by the formation of carbamate (*N*-carboxy-) salts. Several alkaline earth and mercury salts of *N*-carboxy-α-amino-acids have been isolated previously,[1,2] and evidence for the formation of carbamates when alkaline solutions of amino-acids are exposed to carbon dioxide has been obtained from paper ionophoresis studies.[3] Lemieux and Barton have observed, by ^{1}H n.m.r. spectroscopy, the formation of carbamate salts from five α-amino acids and three dipeptides in a carbonate–hydrogen carbonate system.[4]

TABLE 1

Optical rotations and ^{1}H n.m.r. spectra of amino-acids

	Optical rotation (20°; 1 dm; 364 nm)				^{1}H N.m.r. (60 MHz; 33°) [a]				
	$\frac{[K_2CO_3]}{[Amino\text{-}acid]}$ [b]	θ_0 [c]	θ_∞ [d]	$t_{\frac{1}{2}}$/min [e]	$\frac{[K_2CO_3]}{[Amino\text{-}acid]}$	Amino-acid concn. (mol l^{-1}) [f]	δ Values (D_2O) α-H_0 [g]	δ Values (D_2O) α-H_∞ [h]	Δδ (p.p.m.)	% Carbamate
L-Serine	1·0	−0·10	+1·09	46 [j]						
	2·5	−0·07	+1·65	51 [k]	2·5	0·25 and 0·5	3·42 (q)	4·02 (q)	0·60	90
	4·0	−0·07	+1·95	55 [l]						
D-Serine	2·5	+0·07	−1·65	50						
L-Alanine	2·5	+0·27	+0·93	40	2·0	0·5	3·50 (q)	3·88 (q)	0·38	60
L-Arginine	2·5	+1·31	+1·43 [m]	*m*	3·0	0·5	3·21br (s)	*n*	*n*	0
L-Arginine, HCl	3·0	+1·44	+2·33	41	3·5	0·5	3·25br (s)	3·90	0·65	80
L-Aspartic acid	3·0	+0·83	+2·205	15	3·0	0·5 and 1·0	3·73 (q)	4·17 (q)	0·44	80
L-Cysteine	2·5	−0·43	+0·12	25	3·0	0·5	3·55 (q)	3·92 (q)	0·37	60
	3·0	−0·47	+0·15	25						
L-Glutamic acid	3·0	+0·57	+2·02	18	3·5	1·0	3·50 (t)	3·80 (t)	0·30	<90
L-Glutamine	3·0	+0·98	+1·43	18	2·5	0·5	3·37 (t)	3·93 (t)	0·56	80
L-Histidine	2·5	−0·94	+1·67	52	3·0	0·5	3·60 (t)	4·20 (t)	0·60	60 [o]
L-Leucine	2·5	+0·47	+0·91	62	3·0	0·5	3·35 (t)	3·90 (t)	0·65	68
L-Lysine	2·5	+1·16	+1·52	110	3·0	1·0	3·25 (t), [2·81 (t)] [p]	3·85 (t), [3·10(t)] [p]	0·50 (0·29) [p]	15(60) [p]
L-Methionine	2·5	+0·44	+1·52	49	3·0	0·5	3·40 (t)	3·98	0·58	75
L-Phenylalanine	2·5	+0·07	+4·30	35	2·0	0·33	3·58 (t)	4·15 (t)	0·57	70
L-Proline	2·5	−5·70	−5·40	100	3·0	0·5	3·91 (t) [q], [3·16 (q)] [r]	3·91 (t) [q], [3·20 (q)] [r]	<0·02 (0·04) [r]	*s*
L-Valine	2·5	+1·02	+1·46	80	2·5	0·6	3·16 (d)	3·77 (d)	0·61	80
Glycine					2·5	0·5 and 1·0	3·39 (s)	3·59	0·20	90
β-Alanine					2·0	0·5	3·00 (t)	3·24 (t) [t]	0·24 [t]	80

[a] Sodium 3-trimethylsilylpropane-1-sulphonate as internal standard. [b] [Amino-acid] = 0·167 mol l^{-1}. [c] Rotations measured within 3 min of mixing. [d] After *ca.* 24 h. [e] Time (obtained from plots) at which the optical rotation was half the equilibrium value; error ±3 min. [f] These high concentrations were necessary to obtain good spectra. [g] Chemical shift of the α-proton measured within 5 min of mixing. [h] Chemical shift of the α-proton at equilibrium, usually after 24 h. [i] From α-proton signal integrals; error ● 5%. [j] Final pH 8·8. [k] Final pH 10·2. [l] Final pH 10·3. [m] Very small increase after 18 h. [n] No new signals. [o] Value after 1 h. A reliable value could not be obtained after 24 h probably because of exchange of other protons by deuterium. [p] Values for ω-CH_2. [q] This triplet becomes broad and then sharpens again. [r] Values for protons on C-5. [s] Value could not be estimated. [t] Chemical shifts for β-CH_2.

Equations (i)—(iii) represent the equilibrium systems probably present in aqueous potassium carbonate and

$$2K^+ + CO_3^{2-} + H_3^+N{\cdot}CHR{\cdot}CO_2^- \rightleftharpoons H_2N{\cdot}CHR{\cdot}CO_2^- + 2K^+ + HCO_3^- \quad (i)$$

$$K^+ + HCO_3^- + H_3^+N{\cdot}CHR{\cdot}CO_2^- \rightleftharpoons H_2N{\cdot}CHR{\cdot}CO_2^- + K^+ + H_2CO_3 \quad (ii)$$

$$2K^+ + H_2N{\cdot}CHR{\cdot}CO_2^- + H_2CO_3\ [\text{or } CO_2\ (\text{solvated})] + OH^- \rightleftharpoons {}^-O_2C{-}NH{-}CHRCO_2^- + 2K^+ + 2H_2O \quad (iii)$$

show the source of carbon dioxide. An aqueous 0·167M-solution of L-serine containing potassium carbonate (2·5 mol. equiv.) has a pH value of 10·2 If carbon

[1] C. Neuberg, A. Grauer, and M. Kreidl, *Arch. Biochem. Biophys.*, 1955, **58**, 169.

[2] 'The Chemistry of Carbon Compounds,' vol. 1B, ed. E. H. Rodd, Elsevier, Amsterdam, 1952, p. 903.

[3] J. L. Frahn and J. A. Mills, *Austral. J. Chem.*, 1964, **17**, 256.

[4] R. U. Lemieux and M. A. Barton, *Canad. J. Chem.*, 1971, **49**, 767.

dioxide is bubbled through the solution for 2 min the optical rotation increases faster than in the absence of carbon dioxide. This is due to the increased rate of formation of carbamate [equation (iii)]. However, if the passage of carbon dioxide is continued to saturation, then the pH value of the solution drops to *ca.* 8. The optical rotation is then reduced considerably because of the instability of the carbamate at this pH value. If the solution is heated subsequently at 100° to expel the excess of carbon dioxide, the pH rises to *ca.* 10 and the optical rotation increases to the equilibrium value. Equilibrium (iii) is most probably the slowest step because all the other reactions are ionic.

In aqueous potassium carbonate the amino-acids are not completely converted into the corresponding carbamates (see Table 1, last column). The extent of reaction must depend on the pK_a value and nucleophilic nature of the amino-group, steric factors, the pH of the solution, and the concentration of potassium carbonate (*cf.* n.m.r. results below, and ref. 4).

L-Alanine exhibited similar changes in optical rotation in aqueous sodium hydroxide containing carbon disulphide. The formation of dithiocarbamates from α-amino-acids under these conditions is well known.[5] The dithiocarbamate salts are more stable than carbamate salts and readily give *S*-alkyl esters by direct alkylation. In contrast, solutions containing carbamates derived from amino-acids (prepared as above) decompose under direct alkylating conditions and yield only the original amino-acid.

The ^1H n.m.r. spectrum of L-serine in deuterium oxide containing potassium carbonate also revealed a change with time. The quartet for the α-proton at δ 3·42 decreased in intensity while another quartet at lower field (δ 4·02) increased in intensity. Equilibrium was attained overnight, after which the ratio of down-field to up-field signals was *ca.* 9 : 1. The doublet from the methylene protons was barely altered, and there was no apparent exchange of the α-proton with deuterium. All the optically active α-amino-acids studied showed similar shifts (Table 1). Glycine and β-alanine, although lacking a chiral centre, showed similar down-field shifts of the α- and the β-proton signal respectively. These changes are consistent with the equilibria (i)—(iii).

The small and slow changes in optical rotation observed with L-lysine are caused by competition between the α- and ω-amino-groups for carbon dioxide [pK_a values of lysine: 2·04 (CO_2^-), 9·06 (α-NH_3^+), and 10·45 (ω-NH_3^+)].[6] At equilibrium the ^1H n.m.r. spectrum showed that 15% of the amino-acid was in the α-carbamate form and 60% in the ω-carbamate form. In the case of arginine the guanidino-group is much more strongly basic than the α-amino-group {pK_a values of arginine: 2·02 (CO_2^-), 9·04 (α-NH_3^+), and 12·48 [ω-$NHC(NH_2)=NH_2^+$]},[6] and it reacts preferentially with carbon dioxide, *i.e.* no change in optical rotation occurs. Formation of arginine hydrochloride, however, alters the pH, and hence the equilibria, to such an extent that a biscarbamate is formed. L-Proline is unusual in showing only small changes in optical rotation and chemical shift. We are unable to assess the extent of carbamate formation, but there is strong evidence that this occurs (*cf.* ref. 3).

The ^{13}C n.m.r. spectra of serine and glycine were measured in the absence and in the presence of potassium carbonate (Table 2), and are consistent with the above results. The time-dependent formation of carbamate was clearly observed from the changes in chemical shift of the α-carbon atom and from the appearance of a new carbamate carbon signal at *ca.* −97 p.p.m. (see Table 2) which relaxed more slowly than that of the amino-acid carboxy-group carbon atom.

For some α-amino-acids the enhancement in optical rotation on adding potassium carbonate is considerable. This property can be very useful when only small amounts of amino-acids are available, or when their specific rotations are small. The optical rotation enhancements may well be much larger at wavelengths shorter than 364 nm, but this was not investigated. The amino-acids are readily recovered from these solutions by passage through an ion-exchange resin.

TABLE 2

^{13}C (Fourier transform) proton-decoupled n.m.r. chemical shifts [a] (22·63 MHz)

	*C*H	*C*H_2OH	C–*C*O_2^-	N–*C*O_2^-	Solvent
L-Serine	+10·7 [b]	+6·9 [c]	−105·2		D_2O [d]
	+9·7	+3·5	−111·7		[e] D_2O–K_2CO_3
	+8·1	+5·6	−110·2	−96·9	
Glycine	+25·6		−105·1		D_2O [g]
	+23·6		−111·0		[e] D_2O–K_2CO_3 [f]
	+21·3		−110·0	−97·4	

[a] In p.p.m. upfield from external dioxan. [b] Doublet in proton-coupled spectrum (*J* 25 Hz). [c] Triplet in proton-coupled spectrum (*J* 23 Hz). [d] 5·2% Solution. [e] Both species were observed 30 min after addition of K_2CO_3. [f] 2·5 mol. equiv. of K_2CO_3 added per mol. of amino-acid. [g] 7·5% Solution.

The direction of mutarotation seems to be diagnostic for the configuration of the α-amino-acid: in all the examples studied the rotation becomes more positive with L-amino-acids and more negative with D-amino-acids. The change in rotation of amino-acids on going from aqueous to acidic solution has been used for determining configurations for many years.[7] However, this is not applicable to acid-sensitive compounds, for which the observation of mutarotation in potassium carbonate may be particularly valuable.

EXPERIMENTAL

All amino-acids were Fluka ' Puriss ' grade and were used without further purification. Optical rotations were measured on a Zeiss photoelectric polarimeter. The polarimeter tubes (1 dm) were filled with the solution of

[5] K. Ishikawa, K. Achiwa, and S. Yamada, *Chem. and Pharm. Bull.* (*Japan*), 1971, **19**, 912.

[6] D. D. Perrin, ' Dissociation Constants of Organic Bases in Aqueous Solution,' Butterworths, London, 1965.

[7] J. P. Greenstein and M. Winitz, ' Chemistry of the Amino Acids,' Wiley, New York, 1961, ch. 2, p. 46.

amino-acid and potassium carbonate in glass-distilled water, and air was excluded during the measurements until equilibrium was attained. Smooth curves were obtained only when air was excluded. ^{1}H N.m.r. spectra were measured on a Perkin-Elmer R10 spectrometer (60 MHz; 33°); the solutions were not removed from the capped tubes until equilibrium was achieved. ^{13}C N.m.r. spectra were measured on a Bruker (Fourier Transform) spectrometer (22·63 MHz; 27°).

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