Amino-acids and Peptides. Part XI. The Kinetics of the Self-condensation of Glycylglycylglycine Methyl Ester.*

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The velocity of the self-condensation of glycylglycylglycine methyl ester in methanol solution to give pentaglycylglycine methyl ester has been measured at 0°, 25°, and 60°. Second-order velocity constants are found, and the activation energy is calculated to be 5·5 kcal./mole. The frequency factor of the Arrhenius equation is 10^{-1} (l. mole^{-1} sec.$^{-1}$), and the entropy of activation is −63 e.u. The possible significance of these abnormally low values is briefly discussed.

Fischer (*Ber.*, 1906, **39**, 471) observed that when solid glycylglycylglycine methyl ester is heated at 100° self-condensation occurs to give pentaglycylglycine methyl ester:

$$2NH_2\cdot CH_2\cdot CO\cdot NH\cdot CH_2\cdot CO\cdot NH\cdot CH_2\cdot CO_2Me \longrightarrow NH_2\cdot CH_2\cdot CO\cdot[NH\cdot CH_2\cdot CO]_4\cdot NH\cdot CH_2\cdot CO_2Me + MeOH$$

Pacsu and Wilson (*J. Org. Chem.*, 1942, **7**, 117, 126) noted that the reaction proceeds in methanol solution at room temperature, the hexapeptide ester separating out; they also examined the products obtained by heating solid glycylglycylglycine methyl ester for longer periods, further condensation then occurring. The self-condensation of D-alanylglycylglycine methyl ester was reported by Fischer (*Ber.*, 1906, **39**, 2893), and of its racemate by Pacsu and Wilson (*loc. cit.*), who made similar observations on DL-leucylglycylglycine. Curtius (*Ber.*, 1904, **37**, 1300) had earlier reported that triglycylglycine ethyl ester behaves in an analogous fashion, but Fischer (*loc. cit.*) found no change on heating either the ethyl or the methyl ester of triglycylglycine at 100°. Recently, Sluyterman and his co-workers (*Rec. Trav. chim.*, 1952, **71**, 137, 277) reported that the latter ester gives apparently polymeric products when heated for a long time alone or in methanolic solution at 100°, but in this case and in that of glycylglycylglycine methyl ester they found evidence of *N*-methyl derivatives in the product.

A preliminary examination with N. H. Woodbury (Thesis, Bristol, 1948) suggested that the kinetics of the self-condensation of glycylglycylglycine methyl ester in methanolic solution had interesting and unusual features; the reaction proceeds slowly at room temperature, and yet the temperature coefficient of the velocity appeared to be remarkably low. We now report the results of a detailed investigation of the kinetics of this reaction.

For the first preparation of the starting material, we coupled benzyloxycarbonylglycyl chloride with glycylglycine methyl ester; hydrogenation of the product gave glycylglycylglycine methyl ester hydrochloride, from which the ester was liberated as required by the addition of a calculated amount of sodium methoxide. A more convenient route was adopted later, using the mixed-anhydride method of Wieland and Bernhard (*Annalen*, 1951, **572**, 190), Boissonnas (*Helv. Chim. Acta*, 1951, **34**, 874), and Vaughn and Osato (*J. Amer. Chem. Soc.*, 1951, **73**, 3547). By the action of ethyl chloroformate in the presence of triethylamine, benzyloxycarbonylglycine was coupled with glycine methyl ester; hydrolysis of the product gave benzyloxycarbonylglycylglycine, which was coupled again with glycine methyl ester; the remaining stages are indicated above.

In the initial kinetic studies we took advantage of the insolubility of pentaglycylglycine methyl ester in methanol, and measured the rate of the reaction by weighing the separated product. A more satisfactory procedure was used in the later work; the reaction mixture was added to aqueous boric acid, and the free amino-groups were then titrated with 0·01N-acid. Control experiments showed that under these conditions both glycylglycyl- and pentaglycyl-glycine esters were titrated. The results reported here were obtained by this method. The velocity of the reaction was measured at 25° with three different initial

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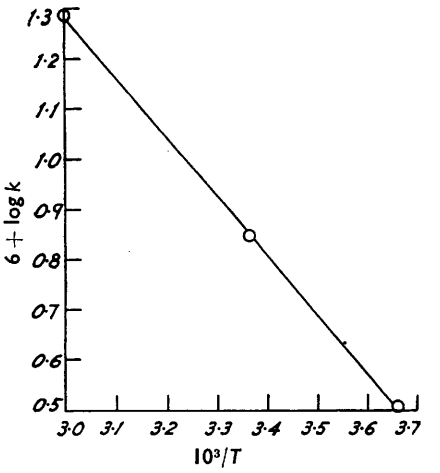
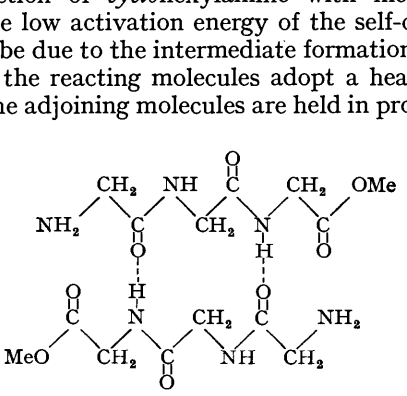
concentrations, and also at 0° and 60°. The prolonged action of boiling methanol on pentaglycylglycine methyl ester failed to yield methanol-soluble products, the reaction being irreversible under these conditions. In view of the work of Sluyterman and his co-workers (*locc. cit.*), a careful examination was made of the hexapeptide ester formed in our experiments. A portion was hydrolysed with hydrochloric acid, and the product was chromatographed on paper, with phenol saturated with water as the mobile phase. With this solvent, sarcosine and glycine are widely separated, and control experiments showed that 1% of sarcosine in glycine could readily be detected. No sarcosine could be found in the hydrolysis product. It may be added that a similar examination of the self-condensation product of triglycylglycine methyl ester in methanol at 60° failed to detect *N*-methylation.



The results of the velocity measurements are shown in Table 1, and it is seen that satisfactory velocity constants are obtained from the second-order equation. The order of the reaction is confirmed by the variation of the time required for partial reaction when the initial concentration is varied (Table 2). From the Arrhenius equation, the energy of activation is calculated to be 5·5 kcal./mole, and the frequency factor A is 10^{-1} (l. mole^{-1} sec.$^{-1}$). From the equation $A = \boldsymbol{RT}e^{\Delta S^*/\boldsymbol{R}}/\boldsymbol{Nh}$ the entropy of activation ΔS^* is calculated to be −63 e.u. Experimental difficulties, particularly at the lower concentrations, prevented the attainment of high accuracy, but there appears to be no doubt of the order of these figures.

The characteristics of this reaction merit some discussion. The frequency factor is very low; Bell (*J.*, 1943, 629) gives the normal range as 10^2—10^7 for second-order organic reactions between uncharged molecules in solution. The correspondingly low entropy of activation must be expected for a reaction which proceeds with measurable rate at normal temperatures with such a small activation energy. Such figures as are available show that the activation energy of the aminolysis of esters is normally considerably higher; Baltzly, Berger, and Rothstein (*J. Amer. Chem. Soc.*, 1950, **72**, 4149) indicate a range of 11—13 kcal./mole, and in experiments to be published we have found an activation energy of *ca.* 14 kcal./mole for the reaction of *cyclo*hexylamine with methyl phenylacetate in methanol. We suggest that the low activation energy of the self-condensation of glycylglycylglycine methyl ester may be due to the intermediate formation of a hydrogen-bonded complex, such as (I), in which the reacting molecules adopt a head-to-tail position; the ester and the amino-groups of the adjoining molecules are held in proximity, and conditions

(I)



would then be favourable for the reaction to be completed by the elimination of methanol. It may be shown that if this hypothesis is correct then in the present case the apparent activation energy would be lower than that for a similar reaction not involving such an intermediate, by an amount equal to the heat of formation of the hydrogen bonds.

A recent paper by Wetzel, Miller, and Day (*J. Amer. Chem. Soc.*, 1953, **75**, 1150) reports low values for the activation energy and activation entropy of the reaction of methyl acetate with ammonia in mixed solvents, but an earlier paper of the same series (Gordon, Miller, and Day, *ibid.*, 1948, **70**, 1946) gives an activation energy of 12·7 ± 2 kcal./mole

TABLE 1.

Temp., 0°. *Initial concn.*, 0·373M.

Time, hr.	19·75	44·0	58·5	82·5
Self-condensation, %	7·8	15·6	19·8	26·2
10^5k, l. mole^{-1} sec.	0·319	0·313	0·313	0·320

Mean, $k_0 = 0{\cdot}32 \times 10^{-5}$ l. mole^{-1} sec.$^{-1}$.

Temp. 25°; (a) *Initial concn.*, 0·0846M.

Time, hr.	23	47	92	158	206	254	300
Self-condensation, %	4·1	8·1	14·2	22·2	27·9	32·6	36·4
10^5k, l. mole^{-1} sec.$^{-1}$	0·604	0·615	0·589	0·596	0·619	0·627	0·628

(b) *Initial concn.*, 0·185M.

Time, hr.	17	38	65	88	102
Self-condensation, %	8·3	16·6	25·9	32·4	35·2
10^5k, l. mole^{-1} sec.$^{-1}$	0·804	0·795	0·812	0·821	0·798

(c) *Initial concn.*, 0·375M.

Time, hr.	2	4·5	8	15·5	23	39·5
Self-condensation, %	1·8	4·1	6·8	12·3	18·2	27·4
10^5k, l. mole^{-1} sec.$^{-1}$	0·687	0·704	0·680	0·671	0·720	0·707

Mean, $k_{25} = 0{\cdot}70 \times 10^{-5}$ l. mole^{-1} sec.$^{-1}$.

Temp., 60°. *Initial concn.*, 0·371M.

Time, hr.	0·75	4·5	8·5	11·0	19·75	24·75	29·0
Self-condensation, %	1·8	9·7	18·4	22·6	35·1	40·2	42·9
10^5k, l. mole^{-1} sec.$^{-1}$	1·88	1·78	1·99	1·99	2·05	2·03	1·94

Mean, $k_{60} = 1{\cdot}95 \times 10^{-5}$ l. mole^{-1} sec.$^{-1}$.

under apparently identical conditions, and a clarification of the position must be awaited. We are now examining the behaviour of glycylsarcosylsarcosine methyl ester, in which hydrogen bonding between peptide groups is excluded, and other analogous reactions, and a detailed discussion is postponed until these results are presented.

EXPERIMENTAL

Glycylglycylglycine Methyl Ester.—The free ester was liberated from the hydrochloride immediately before use. To a suspension of the hydrochloride in dry methanol was added methanolic sodium methoxide (0·93 equiv. calculated on the determined chloride content of the salt), the temperature being kept at −5°. The solvent was removed rapidly below 30°, and the residual solid was extracted with warm dry chloroform. The extracts were filtered and most of the chloroform was removed *in vacuo* at room temperature. Dry ether was added, and the crystalline ester was filtered off, washed with ether, and dried in a vacuum-desiccator for 15 min.

Kinetic Measurements.—Procedure. Glycylglycylglycine methyl ester was dissolved in sufficient methanol (dried with magnesium) to give a solution of approximately the required concentration; centrifugation was usually needed to give a clear solution. Approximate volumes of the solution (0·1—0·5 ml.) were then delivered by means of a micropipette into numbered, weighed test-tubes (3 × $\frac{3}{8}$ in.). The tubes were sealed and then weighed again (the weight of the drawn-off end being included). The tubes were placed in a thermostat and at suitable intervals they were removed, broken into 2% aqueous boric acid (5 ml.), and titrated with 0·01N-hydrochloric acid, with, as indicator, 4 drops of a mixture of bromocresol-green and methyl-red (5 : 2 vol. of 0·1% solutions in ethanol). It was sometimes necessary to warm the solutions slightly before titration to hasten the dissolution of pentaglycylglycine methyl ester.

Results. The results are shown in Table 1. At the highest concentration (0·37M), some condensation occurred before the first titration could be made, and the figures recorded have been corrected for the time taken in making up and weighing the solutions, the true initial concentration being obtained by extrapolation. The velocity constants, k, are derived from the second-order equation.

The times for 30% reaction at 25° were obtained graphically from the results recorded

above, and Table 2 shows the apparent order of the reaction, n, calculated from the equation $n = 1 + \frac{\log t_1/t_2}{\log a_2/a_1}$, where t_1 and t_2 are the times for partial reaction at initial concentrations a_1 and a_2 respectively.

TABLE 2.

Initial concn., mole/l.	0·0846	0·375	0·185	0·375
Time for 30% reaction, hr.	225	43	80	43
Apparent order of reaction, n	2·1		1·9	

From the gradient of the graph shown, the Arrhenius equation gives an activation energy of 5·47 kcal./mole, and a frequency factor A of 10^{-1} (l. mole^{-1} sec.$^{-1}$). The corresponding entropy of activation ΔS^* is calculated to be -63 e.u.

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