175. *Glycine Peptides. Part I. The Polymerization of Piperazine-2 : 5-dione at 180°.*

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When piperazine-2 : 5-dione is heated with water at 180° a polymer of glycine is formed. The polymer appears to exist in equilibrium with the dione in solution, but the equilibrium is continuously disturbed by hydrolysis and decomposition of the dione. It is estimated that, for the reaction piperazinedione(solid) $\longrightarrow$ polymer(solid), $\Delta F = -260$ calories.

Higher peptides of glycine can be prepared in various ways. They are obtained by condensation of the methyl or ethyl esters of the tripeptide (Fischer, *Ber.*, 1906, **39**, 471; Abderhalden and Fodor, *Ber.*, 1916, **49**, 567; Pacsu and Wilson, *J. Org. Chem.*, 1942, **7**, 117), but not of the tetrapeptide (Fischer, *Ber.*, 1906, **39**, 2927; Pacsu and Wilson, *loc. cit.*; Sluyterman and Veenendaal, *Rec. Trav. chim.*, 1950, **69**, 858). Anhydro-*N*-carboxyglycine polymerizes in the presence of water, with loss of carbon dioxide, to form polymers of glycine (Leuchs, *Ber.*, 1906, **39**, 857), and this reaction has been widely applied. When glycine is heated in glycerol to 170—180°, there are formed piperazine-2 : 5-dione, glycine tetrapeptide, and other glycine polymers, and prolonged reaction affords only polymers (Maillard, *Ann. Chim.*, 1914, **1**, 519; 1914, **2**, 210). Recently, Polyakova and Vereschagin (*Doklady Akad. Nauk*, 1949, **64**, 607) have shown that if piperazine-2 : 5-dione is heated in water at 170° under 200—4000 atm., most of it is hydrolysed, but about 25% is converted into an insoluble polymer of glycine.

The present paper deals with the preparation of glycine polymers from piperazine-2 : 5-dione in water at 180°. The use of high pressures is not necessary. The results of a number of experiments are given in Table 1. It will be seen that the yield of polymer depends on the time of heating and the ratio of water to dione. The presence of water is necessary; in its absence the reaction proceeds slowly, if at all. The optimum proportion is 0·5—1 part of water to 1 part of dione. Amounts in excess of this do not accelerate the reaction, and the yield of polymer is reduced. Less water gives a discoloured product. No polymer is formed if this proportion exceeds 3 : 1 (Table 1, 14—17). The formation of polymer is a fairly rapid reaction, the maximum yield being reached in 3—6 hours. Beyond this time the yield slowly declines owing to secondary reactions (Table 1, 6—11), *viz.*, (1) hydrolysis of dione to glycylglycine and glycine, and (2) an irreversible decomposition of glycine to ammonium carbonate and compounds which liberate ammonia with alkali, possibly ammonium glycollate. Traces of methylamine also seem to be present.

Reaction (2) becomes significant after more than 6 hours' heating, so a simple analysis of the liquid in contact with the polymer becomes impossible.

A few experiments were carried out in solvents other than water. Saturated sodium chloride solution (18) gave a somewhat lower yield of polymer; otherwise the results were similar to those obtained with water. Saturated calcium chloride solution (40—45%) gave entirely different results, only traces of polymer being formed; the product, when warm, was a viscous liquid, which solidified on cooling, and dissolved almost completely in water. With glycol the reaction product was very dark, and had obviously decomposed; it contained much unaltered dione, and only traces of polymer. With 90% glycol and 10% water the result was similar.

No polymer could be obtained by heating glycine with water. The results of these experiments are shown in Table 2. The proportions of water to glycine in 1—4 are equivalent to those of water to dione in 6—11 of Table 1. The irreversible decomposition of glycine appeared to be much greater than that of dione under similar conditions, for half the glycine nitrogen was present as ammonium salts after 18 hours, and about a quarter as ammonium carbonate, volatile in steam.

TABLE 1.

1	2	3	4	5	6	7	8	9	10	11	12	13
								N in solution, %:				
Expt. no.	Dione, g.	H_2O, g.	H_2O/N, mol.	Time, hr.	Polymer, %	N in solution, %	N Balance, %	Dione N	Non-dione N	Volatile N	Amino-N	*n*
1	3·8	0·8	0·67	3	5	94	−1	88	6	—	3	2·0
2*a*	3·8	0·8	0·67	18	72	24	−4	—	—	—	—	—
2*b*	3·8	0·8	0·67	18	72	—	—	—	—	—	—	—
3	5·0	2·5	1·59	3	73	27	0	10	17	—	11	1·55
4*a*	3·8	1·9	1·59	18	58	—	—	—	—	—	—	—
4*b*	3·8	1·9	1·59	18	61	42	+3	—	—	—	—	—
5*a*	3·8	1·9	1·59	30	37	—	—	—	—	—	—	—
5*b*	3·8	1·9	1·59	30	40	25	−35	—	—	—	—	—
6*a*	5·0	5·0	3·17	1·5	29	70	−1	54	16	—	8	2·0
6*b*	5·0	5·0	3·17	1·5	30	70	0	55	15	—	8	1·9
7	5·0	5·0	3·17	3	49	51	0	18	33	—	24	1·4
8	5·0	5·0	3·17	6	60	40	0	18	22	—	18	1·22
9	3·8	3·8	3·17	18	50	51	+1	22	20	9	17	1·18
10	3·8	3·8	3·17	30	40	60	0	—	—	—	—	—
11	3·8	3·8	3·17	68	40	52	−8	—	—	—	—	—
12*a*	3·8	5·7	4·76	3	24	75	−1	28	47	4	31	1·4
12*b*	2·0	3·0	4·76	3	23	77	0	—	—	—	16	—
13*a*	3·0	6·0	6·34	3	13	87	0	—	—	—	—	—
13*b*	3·8	7·6	6·34	3	10	89	−1	35	54	4	34	1·47
14	3·0	9·0	9·51	3	2	98	0	53	45	—	27	1·7
15	1·0	5·0	15·9	3	0	—	—	—	—	—	—	—
				24	0							
16	1·0	10·0	31·8	3	0		Some dione crystallized					
				24	0		With Ph·CO·Cl, 0·7 g. of hippuric acid					
17	1·0	20·0	63·4	3	0		Some dione crystallized					
				24	0							
18	3·8	3·8 *	—	3	34	65	−1	40	25	—	13	1·9
19	3·8	3·8 †	—	1·5	4	89	−7	14	75	—	19	4·0
20	3·8	3·8 †	—	3	3	91	−6	8	83	—	32	2·6
21	3·8	3·8 ‡	—	3	0		Solution very dark and decomposed					
22	6·6 §	4·8	3·17	3	18	79	−3	14	65	—	43	1·5
23	1·0 ‖	2·0	6·34	3	40	—	—	—	—	—	—	—

* 3·8 ml. of saturated sodium chloride solution.
† 3·8 ml. of saturated calcium chloride solution.
‡ 3·8 ml. of glycol. § 6·6 g. of glycylglycine. ‖ 1 g. of polymer.

No polymer was formed when glycine tripeptide (1 g.) was heated with water (2 g.) for 3 hours at 180°. On the other hand, glycylglycine formed polymer (Table 1, 22).

In all cases where polymer was formed, the dilute aqueous solution obtained after separation of the polymer gave a yellow colour with amminonickel hydroxide solution, showing the presence of soluble peptides, and was often turbid. Glycine, glycylglycine, and piperazine-2 : 5-dione give no visible reaction with this reagent. The solutions obtained by heating glycine with water also gave no reaction, indicating that peptides higher than glycylglycine were absent.

The interpretation of the experimental results is difficult owing to the complexity of reactions taking place. The following scheme seems most probable. (1) Piperazinedione is hydrolysed to glycylglycine. (2) Successive molecules of dione add on to the terminal amino-group of glycylglycine, building up an insoluble polymer; during this stage the amount of polymer is increasing. (3) Glycylglycine is hydrolysed to glycine. (4) When, because of reactions (1) and (3), the concentration of dione in the aqueous phase falls below a certain value, reaction (2) is reversed: the amount of polymer decreases. (5) Glycine decomposes to ammonium carbonate and other products.

Consider the system polymer–dione–water. It may be assumed that the polymer is a mixture of products of varying chain length, but all capable of being accommodated in the crystal lattice of polyglycine, and forming a single solid phase. This is the case both with natural polymers, such as cellulose, and with artificial polymers such as Nylon. If dione and polymer were in equilibrium with each other through the aqueous phase, they would form a system of two components, dione and water, with three phases (polymer, aqueous solution of dione, and water vapour) and therefore having one degree of freedom. At any temperature the composition of the solid and liquid phases would be fixed, and would be independent of the proportions in which they were present, provided both were present. It would follow that the average chain length of the polymer and the concentration of dione in the liquid at equilibrium would be determined by the temperature only.

It is not possible to test rigorously whether an equilibrium does exist between dione and polymer, because it is not possible to prevent the steady hydrolysis of both these to glycine; but it is obvious that a certain concentration of dione is necessary to bring about polymer formation (Table 1 : 3, 7, 12—17), and it is reasonable to assume that polymer formation will cease when the dione concentration falls to this value, because of either conversion into polymer or hydrolysis to glycine.

TABLE 2.

Expt. no.	Glycine, g.	Water, g.	Mol.-ratio *	Time, hr.	Total N, %	Volatile N, %: with alkali	in steam
1	7·5	3·9	3·17	3	98	—	—
2	7·5	3·9	3·17	24	97	—	—
3	5·0	2·6	3·17	67	94·3	72·5	40·4
4	5·0	2·6	3·17	18	94·0	53·3	23·7
5	1·0	2·0	8·33	3	—	—	—
6	1·0	20·0	83·3	3	—	—	—

* Mol. ratio = free + combined water/glycyl residues (·NH·CH$_2$·CO·).

TABLE 3.

Expt. no.	1	3	6*a*	6*b*	7	8	9	12*a*	13*b*	14
Time, hr.	3	3	1·5	1·5	3	6	18	3	3	3
Polymer, %	5	73	29	30	49	60	50	24	10	2
C_p	(420)	20	(54)	(55)	18	18	22	18·7	17·5	17·7

Mean of 3, 7, 8, 9, 12*a*, 13*b*, 14 : 18·8.

Similarly, it is not possible to test for constant average chain length, for here, too, hydrolysis interferes. The chain length of these polymers will be the subject of another paper, but preliminary measurements indicate that it continues to grow after the total weight of polymer has reached a maximum, and even, owing to hydrolysis, is beginning to decline. Although this increase in chain length involves the elimination of water, the amount would not be sufficient to affect the composition of the aqueous phase appreciably, and the concentration of dione in equilibrium with the polymer at a particular temperature would not differ appreciably from the true equilibrium value for that temperature, even though the polymer had a chain-length distribution different from the equilibrium value. The principal free-energy change in a polymerization reaction is for the conversion of monomer into polymer; the differences in free energy between chains of different length are small.

The concentration of dione in the solutions in contact with the polymers can be calculated from the data in Table 1. It is assumed that all the water in the system is present in the aqueous phase, and none in the polymer. This is only approximately correct. The water present is taken as that given in col. 3, and the percentage of the original dione present is given in col. 9. Strictly, the available water should be corrected for the small amount consumed in forming the free amino-groups (col. 12). The accuracy of the data, however, scarcely justifies such a refinement, and it does not materially affect the results, which are shown in Table 3. It will be seen that, with the exception of experiments 1, 6*a*, and 6*b*, in which the reaction was incomplete, the concentration of dione lies between 17·5 and 22 g. per 100 g. of water, whereas the amount of polymer formed varies from 73% to 2% of the dione originally present.

It seems that an approximate equilibrium is fairly rapidly established between solid polymer and dione in solution, and at this point polymer formation ceases. The balance is continuously disturbed by reactions (1), (3), and (5), the last of which is certainly irreversible. If the concentration of dione in equilibrium with polymer is C_p, and if the saturation concentration of dione at the same temperature is C_s, then for the reaction dione(solid) ⟶ polymer(solid), we have

$$\Delta F = RT(\ln C_p - \ln C_s).$$

The approximate value of C_p at 180° from Table 3 is 188 g. per 1000 g. of water. No data are available on solubilities of piperazine-2 : 5-dione at high temperatures: those measured at 20° and 100° are 14·2 g. and *ca.* 100 g., respectively, per 1000 g. of water, and extrapolation to 180° gives a value for C_s of 250 g. per 1000 g. water. From this it follows that for the transformation of solid dione to solid polymer at 180°, $\Delta F = -260$ calories approximately.

The polymer is the stable solid phase, into which the solid dione will be transformed at 180°, but the margin of stability is small, and at other temperatures the situation might be reversed. At 140° polymer is still the stable solid phase.

The data in Table 1 do not provide a basis for any firm conclusions as to the equilibrium between dione and glycylglycine, or between glycylglycine and glycine. The fact that polymer is formed from glycylglycine would appear to indicate that the equilibrium mixture of glycylglycine and dione contains a substantial proportion of dione at 180°. To give 18% of polymer at least 36% must have been converted into dione, and this would be in agreement with the ease with which glycylglycine and its derivatives can be converted into dione. The " degree of polymerization of the soluble peptides," *n*, col. 13 of Table 1, shows a tendency to decrease to 1 as the reaction time is increased, indicating that the equilibrium between glycylglycine and glycine lies on the glycine side.

EXPERIMENTAL

The polymerizations were carried out in sealed glass tubes in a Carius furnace fitted with a thermoregulator. The reactions were timed from when a thermometer in contact with the iron protecting casing showed a temperature of 170°. At the end of the heating period the tubes in their protecting cases were taken from the furnace and allowed to cool to room temperature (about an hour). In the tubes heated for 18 hours or more there was a crystalline sublimate, probably ammonium carbonate, and those heated for 30—70 hours showed some pressure when opened.

The contents of the tubes were boiled with 250 ml. of water, during which ammonia was lost in some cases (Table 1, 5*b*). The solutions were set aside for 24 hours, and the polymer was then separated by centrifuging, washed once with 50 ml. of water, and once with 50 ml. of methanol, and dried *in vacuo* (over $CaCl_2$) or in an air-oven at 70—80°. The washings were added to the centrifuged solution, which was made up to 500 ml. The solutions were analyzed for: (1) total nitrogen (Kjeldahl); (2) nitrogen evolved on heating with alkali, *i.e.*, ammonia and ammonium salts, 10 ml. of the solution being distilled with 10 ml. of 2N-sodium hydroxide in the Kjeldahl apparatus; (3) free amino-groups, 10 ml. of the solution and 25 ml. of neutral 20% formaldehyde solution being titrated with N/10-barium hydroxide (with phenolphthalein);

(4) piperazine-2 : 5-dione, 10 ml. of the solution being kept with 2 ml. of 2N-sodium hydroxide for 1 hour at room temperature, then neutralized by hydrochloric acid (methyl-red), and titrated as in (3), twice the increase in the titre giving nitrogen as piperazine-2 : 5-dione.

In the experiments with glycine (Table 2) the contents of the tubes were dissolved directly in cold water and made up to 500 ml. They were analyzed as in (1) and (2) and for nitrogen volatile with steam (ammonium carbonate).

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