THE ACTION OF ALKALIES ON PEPTIDES AND ON KETOPIPERAZINES.

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In a previous communication[1] we reported on the action of weak and of stronger alkali on dextro-alanyl-dextro-alanine anhydride. It was observed that the anhydride was partially racemized if it was allowed to remain in an aqueous solution containing one equivalent of sodium hydroxide for 24 hours. When the concentration of the alkali was increased tenfold, the anhydride was rapidly converted into the dipeptide and the latter preserved its normal optical activity in the course of 72 hours. It was then suggested that, if found to be general, this peculiarity could have a theoretical and a practical value: first, it could be used for the detection of the presence of ketopiperazines in the protein molecule; secondly, it might furnish an explanation as to the mechanism of the racemization of proteins. Dakin was the first to advance a comprehensive theory of this phenomenon in protein. Kossel and Weiss prior to Dakin observed that when proteins are acted upon by half normal alkalies, certain of their component amino acids lose part or all of their optical activity, whereas other amino acids remain with their optical activity unimpaired. Dakin sought to explain this phenomenon by the assumption that only those amino acids are racemized which are situated between two other amino acids. Thus, in a tripeptide, only the central and not the terminal amino acids can be racemized. Dakin further postulated that the intermediate phase in the process of racemization consisted in an enolic tautomerization. Thus, considering the case of glycyl-levo-

[1] Levene, P. A., and Pfaltz, M. H., *J. Biol. Chem.*, 1925, lxiii, 661. All other references are contained in that publication.

alanyl-glycine, the entire process may be represented in the following way.

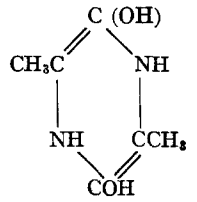
$$CH_2NH_2CO\cdot NHCHCO\cdot NHCH_2COOH \rightarrow CH_2NH_2CO\cdot NHC = C(OH)\cdot NHCH_2COOH$$

$$\underset{\text{levo}}{CH_3} \qquad\qquad\qquad\qquad CH_3$$

$$\rightarrow CH_2NH_2CONHCH(CH_3)CONHCH_2COOH$$

d, l form.

In our previous communication it was shown that ketopiperazines represent a convenient system for enolization and that therefore when exposed to the action of alkali, they may suffer racemization as long as the ring structure remains intact. Thus it was suggested that in the case of alanyl-alanine anhydride the intermediate phase may have the following structure.



As another instance of racemization in a ketopiperazine may be quoted the prolyl-glycine anhydride isolated by Levene and Beatty from the products of tryptic digestion of gelatin. This ketopiperazine was nearly 75 per cent racemized.

To our knowledge, no experimental evidence exists as to the racemization of simple polypeptides. It is therefore expedient to test the influence of dilute alkalies on simple polypeptides and to compare it with that on ketopiperazines. Our views on the mechanism of racemization of protein and on the presence of ketopiperazines in the protein molecule will be greatly influenced by the outcome of such experiments.

In the present communication are reported the results of observations on one tripeptide, namely, glycyl-levo-alanyl-glycine; on one dipeptide, levo-alanyl-glycine, and on two ketopiperazines, namely levo-alanyl-glycine anhydride and levo-prolyl-glycine anhydride. The results of the observations are the following:

Tripeptide.—In dilute alkali (0.1 N) after 48 or 72 hours, there

was observed no hydrolysis of the peptide, no change in its optical rotation, and no change in the optical rotation of the alanine obtained from it. In a 3 per cent concentration in normal alkali in the same interval, there was observed a marked degree of hydrolysis and no trace of racemization of the alanine obtained on hydrolysis of the product of the action of the alkali.

Dipeptide.—In the course of 48 hours, a 1.5 per cent solution of the peptide in 0.1 N alkali remained unchanged with respect to rotation and hydrolysis. The alanine obtained on hydrolysis of the final product of the action of alkali had its original rotation of $[\alpha]_D^{25} = -14.3°$.

The same peptide in the same concentration in solution of N alkali, at the end of 48 hours suffered hydrolysis to the extent of 52 per cent, and the final product of the action of alkali on complete acid hydrolysis furnished alanine with the original rotation of $[\alpha]_D^{25} = -14.9°$. Thus, in the case of levo-alanyl-glycine, no racemization was observed under the conditions of the present experiment.

Ketopiperazines.—Alanyl-glycine anhydride. Three experiments were performed with this ketopiperazine. In one, the concentration of the peptide was 3 per cent in a solution containing 0.4 of an equivalent of alkali; in the second experiment, a 2 per cent solution of the ketopiperazine in a solution containing 0.7 of an equivalent was kept for 48 hours at 15°C. and in the third, the conditions were the same as in the second, except for the temperature, which was somewhat higher, namely, 18°C. In the first instance, the ketopiperazine was hydrolyzed into the dipeptide only to the extent of 30 per cent but no perceptible racemization was observed. The alanine obtained on complete acid hydrolysis had the specific rotation of $[\alpha]_D^{25} = -13.0°$.

In the second experiment at the end of 48 hours, all of the ketopiperazine was converted into the dipeptide and the rotation of the alanine obtained on hydrolysis was $-8.1°$. Finally, in the third experiment, only 72 per cent of the ketopiperazine was converted into the dipeptide and the alanine obtained from it had the specific rotation of $[\alpha]_D^{25} = -5.0°$.

In solutions containing four or eight equivalents of alkali, there was observed considerable hydrolysis also of the dipeptide. The alanine obtained from the final product had the optical rotation of $[\alpha]_D^{25} = -14.0°$.

Levo-prolyl-glycine anhydride.—The material used in these experiments was of the original ketopiperazine obtained by Levene and Beatty from the products of tryptic digestion of gelatin. It had a specific rotation of $[\alpha]_D^{20} = -66°$; hence, it was about 66 per cent racemized. The *l*-proline obtained from it had the specific rotation of −18.4 or was about 75 per cent racemized.

A 2 per cent solution of this ketopiperazine in a little less than one equivalent of alkali showed that after 24 hours 80 per cent of the anhydride was converted into the peptide. The rotation of the proline obtained on hydrolysis of the final material was $[\alpha]_D^{20} = -3.8°$.

The same substance in the same concentration but in a solution of normal alkali showed 96 per cent conversion into the dipeptide and the proline obtained from it had a specific rotation of $[\alpha]_D^{20} = -3.7°$ Thus this ketopiperazine seems to be more resistant towards the hydrolytic action of alkali and hence is racemized by strong as well as by weak alkali.

CONCLUSIONS.

1. The tripeptide glycyl-levo-alanyl-glycine in solution of either one or ten equivalents of alkali does not undergo racemization on standing.

2. The dipeptide levo-alanyl-glycine under the conditions given in (1) does not undergo racemization.

3. In ketopiperazines, levo-alanyl-glycine anhydride and in levo-prolyl-glycine anhydride under the influence of dilute alkalies, racemization takes place.

4. Racemization in the present experiments was never complete. The degree of racemization seems to depend, on the one hand, on the stability of the ketopiperazine ring; on the other, on the concentration of the alkali.

5. The significance of these observations will depend on the outcome of the work on a larger number of polypeptides and ketopiperazines. The work is now in progress in this laboratory.

EXPERIMENTAL PART.

All experiments were carried out in a uniform manner. The initial solution was made with the solvent cooled to about +3°C. The solu-

tion was then kept at a constant temperature of 18°C. In Experiment 9 the temperature was +15°C.

The rotations were measured for a short period at intervals varying from 15 to 60 minutes. These measurements, however, will not be recorded here in view of the fact that they are of little importance without simultaneous measurements of the ratios between the amino and the total nitrogen, which would have involved a great deal of work. At the end of each experiment such measurements were made and therefore both the optical rotations and nitrogen ratios are recorded.

At the end of each experiment, an aliquot part of the solution generally 75 per cent (15 cc. if the original solution was 20 cc.) was diluted with an equal volume of acid of the same normality as that of the alkali used in the experiment. These solutions were used for measurements of the rotations and the nitrogen ratios. From these data the extent of hydrolysis brought about by the alkali can be measured with a fair degree of approximation.

For hydrolysis an aliquot part of the neutral solution was concentrated and made up to a volume of 20 cc. of a solution containing 10 per cent of hydrochloric acid. This solution was heated in a sealed tube at 105°C. for 12 hours. It was found that at the end of that time, the hydrolysis was complete. The concentration of the important amino acid in this solution was calculated from these values.

All rotations were measured at D light in 200.0 mm. tubes. The experimental data are given in the table on the following page.

Name of substance.	(1) Concentration of substance.	(2) Concentration of alkali.	(3) Volume.	(4) α Of original solution.	(5) α At the end of experiment.	(6) After neutralization.	(7) $\frac{NH_2N}{T \cdot N}$ End of experiment.	(8) α After hydrolysis.	(9) T·N in portion hydrolyzed in 20 cc.	(10) $[\alpha]_D^{25}$ active amino acid.
	per cent		*cc.*				*per cent*		*gm.*	
Glycyl-levo-alanyl-glycine.	(1) 3	N/1	20	+2.92°	+1.60°	+2.00°	63.0*	−0.25	0.0742	−14.5°†
"	(2) 3	N/1	20	+3.13	+3.15	+3.80°	42.0	−0.23	0.0742	−13.9
Levo-alanyl-glycine.	(3) 1.5	N/1	20	−0.25	−0.20	−1.08	73.0	−0.17	0.0546	−14.9
"	(4) 1.5	N/10	20	−0.33	−0.30	−1.42	48.5	−0.16	0.0525	−14.3
Levo-alanyl-glycine anhydride.	(5) 3	N/1	20	+0.80						
				+0.95	+0.82	+1.00	65.3	−0.35	0.0826	−13.3
	(6) 3	N/20	20	+0.02	+0.35	+0.36	16.9	−0.35	0.0854	−12.8
"	(7) 1.5	N/1	20	+0.20						
				+0.50	+0.43	+0.50	78.0	−0.17	0.0406	−14.0
"	(8) 2	N/10	30	—	+0.40	+0.32	48.5	−0.25	0.0938	− 8.1
"	(9) 2	N/10	20	−0.14	+0.45	+0.52	37.6	−0.10	0.0588	− 5.0
Levo-prolyl-glycine anhydride.	(10) 2	N/1	20	−0.50	−0.11	−0.32	48.5	−0.08	0.0490	− 3.7‡
"	(11) 2	N/10	20	−1.28						
				−0.82	−0.09	−0.22	40.0	−0.08	0.0476	− 3.8

* The rotation of the alanine obtained on direct hydrolysis of the peptide without preliminary treatment with alkali = $[\alpha]_D^{25} = -14.5°$.

† The specific rotation of the ketopiperazine was $[\alpha]_D^{25} = -66.0°$ (in water). The proline obtained from it on direct hydrolysis had a specific rotation of $[\alpha]_D^{25} = -18.4°$.

‡ All amino-nitrogen estimations were made in Van Slyke's apparatus.