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POLYCONDENSATION OF CERTAIN PEPTIDE ESTERS. II. PROTEIN MODELS. NOTES ON THE PREPARATION OF TRIPEPTIDE METHYL ESTERS[1]

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In the preceding article[3] it was shown that the tripeptide methyl ester of glycine, on being heated, undergoes condensation in a series of successive reactions yielding the 96-peptide methyl ester of glycine as the final product. Application of the procedure to other tripeptide methyl esters has now been undertaken in order to open the way for the preparation of synthetic protein-like substances in accordance with the views postulated by Bergmann's periodicity hypothesis (1, 2, 3). It was hoped that substitution of different amino acids for glycine in the starting materials would lead to model substances not possessing the extreme insolubility of the glycine polypeptide esters. It should be pointed out that once the proper technique for the preparation of these various model substances has been established, synthetic products containing optically active amino acids linked together in known order can be made available for physiological and enzymatic studies.

A search of the literature for suitable starting materials revealed that, whereas several tripeptides had been prepared in pure state, both the methyl ester hydrochlorides and the methyl esters themselves were unknown. The only simple tripeptide methyl ester which had been prepared, other than G_3M,[4] was *l*-alanylglycylglycine methyl ester (4). In undertaking the preparation of such tripeptide esters considerable and unexpected difficulty was encountered for the reasons discussed in the following section. From the products finally obtained, only *dl*-alanylglycylglycine methyl ester (AG_2M) and *dl*-leucylglycylglycine methyl ester (LG_2M) were available in pure form.

I. THE PREPARATION OF METHYL ESTERS OF TRIPEPTIDES

For our experiments we decided to use the methyl esters of *dl*-alanylglycylglycine, *dl*-leucylglycylglycine, *dl*-alanyl-*dl*-leucylglycine (ALGM) and glycyl-*dl*-leucyl-*dl*-alanine (GLAM). We synthesized the free tripeptides in the same general manner in which they were originally obtained by Fischer (5, 6). In these syntheses, as one of the steps, ammonia is employed for the replacement of halogen by an amino group in the respective halogeno peptides, a reaction often resulting in the formation of large amounts of by-products (6). For instance, in

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[3] Paper I, Pacsu and Wilson, *J. Org. Chem.*, **7**, 117 (1942).

[4] See footnote 3, Paper I.

the ammonolysis of α-bromoisocaproylalanine the corresponding diketopiperazine is formed in considerable amount, as well as the hexenic acid derivative of alanine, $(CH_3)_2CH \cdot CH{=}CH \cdot CO \cdot NH \cdot CH(CH_3)COOH$, where hydrogen bromide was split off. The formation of a corresponding hydroxy acid, $(CH_3)_2 CH \cdot CH_2 \cdot CHOH \cdot CO \cdot NH \cdot CH(CH_3)COOH$, is likewise possible under the conditions of the experiment. Finally, the prolonged action of aqueous ammonia may be effective in splitting the peptide bond. It occurred to us that much of the by-product formation may actually take place *after* the amination has been completed, as a result of long contact of the product with strong ammonia. For this reason pilot experiments were run on most of the compounds we desired to aminate before subjecting the main part of the material to the treatment.

TABLE I

TIME NECESSARY FOR COMPLETION OF AMMONOLYSIS AS INDICATED BY HALOGEN ION ESTIMATIONS

SUBSTANCE	TIME (HRS.) AT 50° 9 *N* AMMONIA	TIME (HRS.) USED BY FISCHER, ABOUT 15*N* AMMONIA
α-Bromopropionylglycylglycine	4	0.5 (100°)
α-Bromoisocaproylglycylglycine	6.5	0.5 (100°)
α-Bromopropionylleucylglycine	2	24 (room temp.)
Chloroacetylleucylalanine	1	0.5 (100°)

TABLE II

AMINO NITROGEN CONTENT OF SYRUPS FROM ESTERIFICATION OF TRIPEPTIDES UNDER STANDARD CONDITIONS

SUBSTANCE	% AMINO N (CALC'D METHYL ESTER HYDROCHLORIDE)	% AMINO N (FOUND)
Alanylglycylglycine	5.52	8.62, 8.44
Leucylglycylglycine	4.74	5.94, 5.79
Alanylleucylglycine	4.52	5.42, 5.34
Glycylleucylalanine	4.52	7.28, 7.38

Samples were withdrawn from these pilot runs at known times and analyzed for halogen ion content; the end-point of the replacement reaction was thus ascertained. This technique in every case led to increased yields over those reported by Fischer, under milder conditions. As shown in Table I, the reaction is in general much more rapid than hitherto supposed.

Experiments for the preparation of the methyl esters of these tripeptides were based on the assumption that methyl alcoholic hydrogen chloride, so successfully employed in the case of G_3M and *l*-AG_2M, was the only methylating agent which could be used due to the insolubility of the peptides in organic solvents and the presence of a reactive amino group. Abderhalden (7) has given general rules for esterification, based largely on Fischer's original procedure for the above two tripeptide esters and for the amino acid esters themselves. Using this procedure, modified only by varying the percentage of hydrogen chloride in the esterifying

mixture from 2.5% to saturated, the following discouraging products were obtained after many different experiments. The GLA and ALG gave non-crystallizable syrups. The AG_2 and LG_2 gave syrups from which small amounts of fine needles slowly separated. This crystalline material, after purification, gave melting point and analytical data for methoxyl and chlorine content in perfect agreement with those of glycine methyl ester hydrochloride. Since it appeared from this that the tripeptides had undergone some fission, all syrups were converted into amorphous solids by trituration with dry ether and analyzed for amino nitrogen. The results given in Table II are typical of the products obtained from the various runs and indicate considerable splitting. Neutralization of the syrups with sodium methoxide yielded basic substances from which a volatile fraction, glycine methyl ester, was removed by distillation. From the residues a series of crystalline compounds were isolated. Their properties and analyses indicated that they represented the corresponding diketopiperazine derivatives, necessarily formed from the splitting off of the end-standing glycine residue in each case. We conclude, therefore, that esterification of the tripeptides under standard conditions leads to alcoholysis, resulting in the splitting of the molecules at the point between the end glycine residue and the remainder of the molecule. The resulting dipeptide ester hydrochlorides, when neutralized, rapidly cyclize to the corresponding diketopiperazines, while the free glycine ester is completely removed when the mixture is concentrated *in vacuo*. The results are summarized as follows.

$$\text{Alanyl-glycyl}\vdots\text{glycine} \xrightarrow[\text{HCl}]{\text{MeOH}} \text{AGM}\cdot\text{HCl} + \text{GM}\cdot\text{HCl}$$

$$\text{Leucyl-glycyl}\vdots\text{glycine} \xrightarrow[\text{HCl}]{\text{MeOH}} \text{LGM}\cdot\text{HCl} + \text{GM}\cdot\text{HCl}$$

$$\text{Alanyl-leucyl}\vdots\text{glycine} \xrightarrow[\text{HCl}]{\text{MeOH}} \text{ALM}\cdot\text{HCl} + \text{GM}\cdot\text{HCl}$$

$$\text{Glycyl}\vdots\text{leucyl-alanine} \xrightarrow[\text{HCl}]{\text{MeOH}} \text{LAM}\cdot\text{HCl} + \text{GM}\cdot\text{HCl}$$

It is interesting to note that previous to Abderhalden's general rules the same process of alcoholysis under nearly the same conditions was pointed out by Pribram (8); little has appeared on the subject since that time (9).

The importance of the time factor was now evident, and the easy preparation of G_3M and *l*-AG_2M under Abderhalden's conditions could be attributed to the rapid and easy crystallization of these particular ester hydrochlorides. Therefore our next series of experiments was run using freshly prepared saturated methyl alcoholic hydrogen chloride in order to ensure rapid esterification. The tripeptide was dissolved in this solution and the product *immediately* precipitated with dry ether, or the solution at once taken down *in vacuo*. The resulting substance was repeatedly taken up in methanol and thrown out of solution with dry ether, the liquid layer being decanted in order to remove as much hydrogen chloride as possible. The final product was dried over fresh sodium hydroxide.

In this manner the new pure crystalline hydrochlorides of AG_2M and LG_2M were obtained; the hydrochlorides of ALGM and GLAM would not crystallize although their analyses agreed reasonably well with the calculated values. Possibly this is due to the fact that they are composed of a mixture of diastereomers.

The hydrochlorides were neutralized with sodium methoxide in the usual manner to liberate the previously unknown free esters. Of these, the AG_2M was obtained in pure crystalline form, the pure LG_2M was a white amorphous powder, while the GLAM was at first an oil but turned into a slightly impure solid crystalline mass on standing; the ALGM could not be crystallized or solidified, and was unavailable for condensation experiments.

Experiments involving esterification of other polypeptides, as well as scission of protein molecules by methanolysis are now in progress in this Laboratory.

EXPERIMENTAL

dl-*Alanylglycylglycine methyl ester.* Fischer's procedure (5) for the production of α-bromopropionylglycylglycine was followed, using diketopiperazine and α-bromopropionyl bromide prepared according to Volhard (10). For the amination of this substance Fischer's method was modified as follows. To 1260 cc. of 9 *N* ammonia was added 168.5 g. of the bromo compound, giving a 0.5 molar solution of the substance. Since a pilot run indicated that at 50° under these conditions the bromide ion content reached the maximum in four hours, the amination was conducted in this fashion. The tripeptide was then worked up according to Fischer's procedure; yield, 100 g. or 78%. A second run gave a yield of 80.5%, whereas Fischer reported 57%; m.p. and physical properties were identical with those reported by him. Titration with alkali in aqueous solution, using thymol blue with the addition of three volumes of alcohol: 0.1368 g. required 6.63 cc. of 0.1 *N* alkali; calculated: 6.74 cc.

From the tripeptide the methyl ester hydrochloride was prepared as follows. To 17 g. of the tripeptide was added 170 cc. of a saturated methyl alcoholic hydrogen chloride solution freshly prepared from absolute methanol. The vessel was rotated while cooling until all the substance had dissolved, and the solution was immediately concentrated to dryness *in vacuo* at room temperature. The resulting syrup was repeatedly taken up in a small quantity of methanol and precipitated by the addition of dry ether, the liquid layers being decanted. After drying over solid sodium hydroxide *in vacuo* the syrup completely solidified. Two crystallizations from a methyl alcohol-ether mixture gave clusters of needles; yield, 20.4 g. or 96.2%. The crystals had the m.p. 154–157° (corr. 157–160°).

Anal. Calc'd for $C_8H_{16}ClN_3O_4$ (253.5): OCH_3, 12.22; Cl, 14.00.
Found: OCH_3, 12.24; Cl, 14.05.

For the liberation of the free methyl ester, a solution containing 2% less than the calculated quantity of sodium methoxide was added slowly to 7 g. of the hydrochloride dissolved in the minimum amount of ice-cold methanol. The mixture was immediately concentrated to dryness *in vacuo* at room temperature and the resulting cloudy syrup was extracted at once with several portions of hot ethyl acetate. On cooling the filtered ethyl acetate solution in ice, the ester separated in crystalline form; yield, 4.6 g. Addition of ether to the filtrate gave 0.4 g., a total yield of 83%. The needles melted at 86–88°, and in water solution were strongly alkaline to litmus, showing a positive biuret reaction. Titration of a 0.0959 g. sample using methyl red required 4.30 cc. of 0.1 *N* acid; calculated: 4.42 cc.

Anal. Calc'd for $C_8H_{15}N_3O_4$ (217): OCH_3, 14.28. Found: OCH_3, 14.22.

dl-*Leucylglycylglycine methyl ester.* The free peptide was obtained in the manner reported by Fischer (5). Diketopiperazine was converted to α-bromoisocaproylglycylglycine

by coupling with α-bromoisocaproyl chloride prepared from isoamyl alcohol according to Hass and Marshall (11). For amination to the tripeptide, a pilot run indicated maximum bromide ion concentration after six and one-half hours heating at 50° in 9 *N* ammonia. These conditions were used with excellent result; from 24.5 g. of starting material was obtained 15.6 g. or an 80.4% yield of the pure tripeptide, as compared with Fischer's 63% yield of unrecrystallized product; m.p. and properties indicated the tripeptide to be identical with Fischer's preparation. Conversion of one gram of the tripeptide to the new methyl ester hydrochloride was accomplished in a manner similar to that used for alanylglycylglycine methyl ester hydrochloride. The substance crystallized spontaneously on concentration of the original solution. It was washed with ice-cold methanol and dried over sodium hydroxide. Recrystallization from absolute methanol with the addition of ether gave fine needles; yield, 0.9 g.; m.p. 220–221° (corr. 227–228°) with decomposition.

Anal. Calc'd for $C_{11}H_{22}ClN_3O_4$ (295.5): OCH_3, 10.49; Cl, 12.01.
Found: OCH_3, 10.47; Cl, 11.94.

The free ester was liberated from the hydrochloride by the same procedure as used for the alanylglycylglycine methyl ester. The extraction was made with chloroform and from the solution, on concentration, a clear syrup was obtained. This was taken up in ether, the solution filtered from a trace of insoluble material, and the filtrate was again concentrated. The ester separated as a white solid which was removed by rubbing with dry petroleum ether and dried over sodium hydroxide; m.p. 70°. The substance was fairly soluble in ether.

Anal. Calc'd for $C_{11}H_{21}N_3O_4$ (259): OCH_3, 11.96. Found: OCH_3, 11.78.

Glycyl-dl-*leucyl*-dl-*alanine methyl ester.* The free peptide was obtained according to Fischer's procedure (6). For the conversion of the chloroacetylleucylalanine to the tripeptide the optimum condition was again determined by a pilot run. To 95 cc. of 9 *N* ammonia was added 13.3 g. of the chloroacetyl compound to give a 0.5 molar solution; this was kept at 50° for one hour, after which the solution was worked up according to Fischer. Yield, 10.8 g. or 88% as compared with Fischer's 70%. Molecular weight determination by electrometric titration gave 262.6; calculated, 259. The methyl ester hydrochloride was prepared by the same procedure as described for the two previous ester hydrochlorides. The product was obtained in solid form but could not be recrystallized; yield, 70%.

Anal. Calc'd for $C_{12}H_{24}ClN_3O_4$ (309.5): OCH_3, 10.01. Found: OCH_3, 10.32.

The free ester was obtained in the usual manner described above for the other tripeptide esters. The product was a clear oil soluble in ether; on standing or scratching, it readily crystallized. The crystalline product was only slightly soluble in ether, but could not be recrystallized from this or other solvents, since it always separated out as a slightly impure oil, the process of crystallization being evidently quite slow. Its aqueous solution is strongly alkaline to litmus, but gives only a faint biuret reaction; yield, 77%; m.p. 102–105°.

Anal. Calc'd for $C_{12}H_{23}N_3O_4$ (273): OCH_3, 11.35. Found: OCH_3, 10.90.

The *dl*-alanyl-*dl*-leucylglycine used in the experiments was prepared according to Fischer (6), except that the intermediate α-bromopropionylleucylglycine was aminated according to our new conditions. The usual pilot run indicated the reaction to be complete in two hours at 50° in 9 *N* ammonia; yield, 83.6% as compared with Fischer's 74%; m.p. and physical properties identical with those given by Fischer. Titration of a sample using the glass electrode gave a molecular weight value of 264.4; calculated, 259. Preparation of the ester hydrochloride and free ester from this substance according to the above conditions led only to impure syrupy products.

II. CONDENSATION EXPERIMENTS WITH NEW TRIPEPTIDE METHYL ESTERS

Attempts to prepare the hexapeptide ester from the crystalline AG_2M for use in condensation experiments by the same procedure as used for G_3M gave a different but interesting result. A 10% methyl alcoholic solution of AG_2M

(MeO, 14.28%) remained clear during a period of 24 days standing at room temperature. After this time the substance was precipitated in apparently amorphous form by addition of ether. The methoxyl content of the product was 6.98%, whereas the calculated value for AG_2AG_2M is 7.71%, indicating that, although the condensation does take place, the hexa-, and to some extent the higher peptide esters, unlike the G_6M, are soluble in methanol.

The AG_2M and LG_2M were then submitted to heating experiments, samples being withdrawn at known times for methoxyl determinations. For AG_2M, runs were made at 80°, 100°, and 110°; the results of the methoxyl analyses are given in Table III. With LG_2M, experiments were carried out at 100° and 110°; the

TABLE III

CONDENSATION OF *dl*-ALANYLGLYCYLGLYCINE METHYL ESTER AT DIFFERENT TEMPERATURES

T = 80° ± 1°		T = 100° ± 1°		T = 110° ± 1°		
Time (hrs.)	% OCH_3	Time (hrs.)	% OCH_3	Time (hrs.)	% OCH_3, 1st run	% OCH_3, 2nd run
0	14.28	0	14.28	0	14.28	14.28
1	13.79	0.4	13.10	0.25	12.42	
2	13.47	1	11.96	0.5	12.16	
4	12.67	2	10.88	1	10.58	10.71
7	11.74	4	7.34	1.5	8.80	
12	9.73	6	5.73	2	7.07	7.53
24	7.42	12	4.04	4	4.75	
48	6.34	24	3.47	6	3.69	4.15
72	5.99	48	2.57	12	2.77	
192	5.09	72	2.23	24	2.07	2.19
480	4.39	96	2.00	48	1.54	
		192	1.60	72	1.25	1.19
		312	1.33	96	1.11	0.86[a]
				264	0.70	
				408		0.55
				845		0.43

[a] This value is for 168 hours heating.

results of the methoxyl estimations are shown in Table IV. With the exception of the experiment at 80° on the AG_2M, all the runs were complicated by the fact that LG_2M melts at 70° and AG_2M melts at 86–88°. Consequently it was found necessary to weigh out the samples in advance in small individual tin cups; after heating, the cups with their contents were re-weighed and placed in the methoxyl apparatus. As with G_3M, G_6M, and $G_{12}M$, there occurred a general fall in the methoxyl contents to values fairly close to the calculated methoxyls for the respective 48- and 96-peptide esters. Some indication was obtained in the case of AG_2M at 110° that the condensation may have proceeded beyond the 96-stage; after 408 hours heating, the methoxyl content was 0.55%, while an additional 437 hours heating lowered the value to 0.43% as compared with the calculated value of 0.52%. Though this latter analysis is still within the experimental

error for the 96-peptide ester, it should be remarked that in all probability the condensation from the 96- to the 192-stage would be an extremely slow process.

We were able to obtain excellent confirmation of the validity of the methoxyl analyses in the following manner. A sample of AG_2M was heated at 80° until the methoxyl content was 4.39% (calc'd for the 12-peptide ester, 4.01%). This substance was completely soluble in water. Titration of a 0.1392 g. sample with 0.01 *N* hydrochloric acid, using the glass electrode, gave an end-point value of 18.6 cc.; the calculated value, assuming condensation for removal of the free amino group, is 19.1 cc. Amino nitrogen determinations on the same substance gave values of 2.13% and 1.97%, as compared with the calculated value of 1.98%.

Unfortunately, the washing-out experiments, used so successfully for the quantitative removal of G_3M and G_6M from the condensation products of the

TABLE IV

CONDENSATION OF *dl*-LEUCYLGLYCYLGLYCINE METHYL ESTER AT DIFFERENT TEMPERATURES

T = 100° ± 1°		T = 110° ± 1°	
Time (hrs.)	% OCH_3	Time (hrs.)	% OCH_3
0	11.96	0	11.96
1	10.90	1.5	9.12
2	9.95	3	7.73
4	8.71	5	5.73
11	5.96	9	4.54
27	4.70	24	3.06
50	3.80	72	2.40
96	2.80	168	1.85
217	2.34	264	1.84

earlier experiments, could not be applied as such to these latter products, as the high peptide esters possessed the unexpected property of being soluble in cold water at all stages. A series of experiments on products obtained by heating AG_2M showed the tripeptide ester to be soluble in warm ethyl acetate, and the hexapeptide ester to be insoluble in ethyl acetate but partly soluble in ethanol and completely soluble in methanol. While the unchanged tripeptide ester could be separated quantitatively from the other products by ethyl acetate, the hexapeptide ester could not be completely washed out with ethyl or methyl alcohol without appreciable removal of some products containing lower methoxyl values. Consequently, since recovered portions of hexapeptide ester usually showed lower methoxyl contents than the calculated for the pure hexapeptide ester, it is not possible to exclude the formation of 9-peptide ester on the basis of such experiments. The non-formation of "cyclol-6", however, is indicated by the following data. A sample of AG_2M heated at 80° for twenty-three hours contained 7.13% methoxyl (calc'd for the hexapeptide ester, 7.71%). Washing with warm ethyl acetate gave little loss in weight, indicating a negligible amount

of unchanged AG_2M at this stage. The residue was then boiled five minutes with methyl alcohol, and a small insoluble portion filtered off. This portion had a methoxyl content corresponding to that of a dodecapeptide ester (found: MeO, 4.53; calc'd: MeO, 4.01). From the alcoholic filtrate, on standing at 0°, a small fraction with 6.45% methoxyl value was recovered by filtration. Although this could be interpreted as being a mixture of 32% hexa- and 68% nona-peptide ester (calc'd for nonapeptide ester: MeO, 5.86), it is as likely that it consists of a mixture of hexa- and dodeca-peptide ester, and consequently no conclusion can be drawn on this point. The quantity of this fraction, however, is very small. The filtrate, on addition of ether, gave a large amount of almost pure hexapeptide ester containing 7.44% methoxyl. It is apparent, as in the case of the glycine peptide ester series, that had "cyclol-6" been the product, a substance containing no methoxyl rather than 4.53% should have been obtained.

It was also found that after heating AG_2M until its methoxyl content reached the calculated value for the hexapeptide ester, practically no unchanged AG_2M could be washed out from the condensation product with warm ethyl acetate. On the other hand, heating to a point midway between the calculated values for the tri- and hexa-peptide esters gave a mixture from which very nearly 50% unchanged AG_2M could be recovered by this treatment. This would seem to indicate that, at least in the earlier reactions, the condensation proceeds much more by stages than does the G_3M condensation. That is, when the methoxyl value reaches the theoretical for the hexapeptide ester of AG_2M, there is practically no tri- and very little dodeca-peptide ester present. If this is true of the later phases of the reactions, nearly uniform products could be obtained at the individual stages of the condensation reaction expressed by the formula 3×2^n.

An experiment was also made to determine if the rate of condensation of AG_2M in methanol solution could be influenced appreciably by heat. A sample was refluxed at 65° for four hours and then precipitated in crystalline state by the addition of ether; its methoxyl content was found to have fallen from 14.28% for the tripeptide ester only to 13.42%. The rate is apparently no faster than would have been the case in the solid state at 65°.

The fact that at 100° and 110° the AG_2M and LG_2M were in an intial molten state does not necessarily presuppose a different mechanism of condensation than that characteristic for G_3M, G_6M, and $G_{12}M$, and for AG_2M at 80°. The clear liquids, after two to four hours heating (that is, at the hexapeptide stage) turned into pink-white solids of apparently crystalline nature. In a trial run on another tripeptide ester, glycyl-*dl*-leucyl-*dl*-alanine methyl ester, a sample was heated at 80° for 114 hours. The methoxyl content dropped from the calculated value of 11.35% for the tripeptide ester to 9.06%. Inasmuch as the calculated value for the hexapeptide ester is 6.03%, a comparison with the results shown in Tables III and IV indicates that the condensation of this substance is very slow. No further experiments with this ester have been performed as yet.

The various polypeptide esters prepared during these condensations are white or slightly colored, apparently amorphous powders, all of which possess the surprising and valuable property of being easily soluble in cold water, in contrast to

the insoluble peptide esters of glycine. The higher peptide esters are insoluble in organic solvents. All give very strong biuret reaction. The importance of utilizing this procedure for the production of model substances is obvious. For instance, we believe that Bergmann's hypothesis of repeating amino acid units in the proteins finds the best synthetic approach in the almost numberless possibilities of order and arrangement which this method makes available. In this introductory paper alone compounds up to molecular weights of about 7000 containing long chains of repeating units such as —A·G·G·A·G·G·A·G·G·A·G·G·A·G·G·A·G·G·— and —L·G·G·L·G·G·L·G·G·L·G·G·L·G·G·L·G·G·— have been reported. The preparation of such substances by the use of Fischer's original method, included in almost every textbook on protein chemistry as the classical method for this approach, would have been a near impossibility.

Molecular weight determinations and other physico-chemical investigations on the water-soluble condensation products are to be carried out in this Laboratory and application of the condensation reaction to other tripeptide esters is being continued.

SUMMARIES

I

Tripeptides with glycine constituents used in this investigation suffer methanolysis under conventional conditions for esterification. The products are dipeptide methyl ester hydrochlorides and glycine methyl ester hydrochloride. Neutralization yields the corresponding diketopiperazines and glycine methyl ester.

A simple procedure is given for the preparation of the tripeptide methyl esters.

II

It has been shown that *dl*-alanylglycylglycine methyl ester and *dl*-leucylglycylglycine methyl ester, on being heated, undergo condensation in a series of successive reactions apparently according to the formula 3×2^n. The course of the reaction has been followed by methoxyl estimations. In the case of AG_2M the final product very likely represents the 96-peptide ester.

From the results of the quantitative analyses of the condensation products of AG_2M it has been concluded that "cyclol-6" is not formed in the reaction. No definite conclusion could be reached as to the formation or non-formation of a nonapeptide ester.

The substances are soluble in water and all give strong biuret reactions.

It has been pointed out that by this condensation reaction the way is open for the preparation of long-chain, high molecular weight protein models in agreement with the recurring structures postulated by Bergmann as existing in all proteins.

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