carried out on substance no. 1 and no. 7, prove that the high poly-condensation products are quantitatively built up of glycine units linked by peptide bonds. The presence of the latter is also indicated by the positive biuret reaction.

(3) The average chain length of the glycine polymers was ascertained by quantitative alkoxy determinations, as in the series of polymer homologs the alkoxy content varies distinctly with growing chain length. NH_2 determinations, although also indicative of the chain length, could not be carried out here owing to the insolubility of the glycine polymers.

(4) The results of alkoxy determinations were throughout in agreement with total nitrogen determinations (Kjeldahl). Moreover, as will be shown in the following paper, in the case of similar alanine poly-condensation products, soluble in water, the results of additional amino group determinations were throughout in very satisfactory agreement with those of alkoxy determinations and fully confirmed the conclusions drawn from the latter as regards the chain length.

Carbon and hydrogen determinations are not suitable means to assess the chain length; the differences in their values for the higher homologs lie within the experimental error.

It appears that high polymers can be obtained more easily and in better yields from the methyl ester than from isobutyl or the ethyl ester of glycine.

Our linear synthetic glycine products resemble in some properties the poly-amides obtained by Carothers[15] by the poly-condensation of di-amines and di-carboxylic acids.

Summary

On condensation under various conditions, glycine ethyl ester yielded a series of water insoluble polymers. The preparations were amorphous and contained ethoxyl in amounts corresponding, respectively, to 12-, 13-, 16-, 17- and 20-glycine peptide ethyl esters. From glycine methyl ester analogous preparations were obtained that contained methoxyl corresponding to 18-, 27- and 30-glycine methyl esters, respectively. Glycine isobutyl ester yielded a product of which the isobutoxy content corresponded to 16-glycine isobutyl ester.

On being heated to 130°, several of these products underwent further polymerization as indicated by decrease in alkoxy content. Preparations that corresponded in composition to 42-glycine ethyl ester and 110-glycine methyl ester were thus secured.

On being subjected to hydrolysis with acid, several of these polymers gave high yields of glycine suggesting that the polymers are in fact polypeptide esters. It appears that high polymers are more easily obtained from glycine methyl ester than from glycine ethyl and isobutyl esters.

The poly-condensation products described are (except the amorphous 12- and 13-glycine ethyl esters) horn-like, practically insoluble in water, in which they show characteristic swelling. They give positive ninhydrin and biuret reactions.

JERUSALEM, PALESTINE RECEIVED DECEMBER 19, 1941

[CONTRIBUTION FROM THE LABORATORY OF HIGH MOLECULAR CHEMISTRY, THE HEBREW UNIVERSITY]

Poly-condensation of α-Amino Acid Esters. II. Poly-condensation of Alanine Ethyl Ester[1]

BY MAX FRANKEL AND EPHRAIM KATCHALSKI

Alanine ethyl ester is a stable compound as compared with glycine ethyl ester. According to Fischer[2] and more recent literature, condensation to a peptide ester giving the biuret reaction has never been observed; moreover, even the formation of alanine anhydride, the so-called lactimide, takes place slowly.

Thus it seemed less probable that alanine ethyl ester would give polymers. We tried therefore to establish experimental conditions specially favorable to intermolecular reaction. Thus, although we obtained clear indications for poly-condensation of alanine ethyl ester on using experimental conditions similar to those described

(1) Certain minor errors in the manuscript as originally submitted were noted by the Editorial Board. Ordinarily these would have been brought to the attention of the authors prior to publication. International conditions at present are such that it appears impossible to follow this procedure except at the risk of indefinite postponement. The Editor has therefore taken the responsibility to make any corrections which appeared to be unquestionably required.—THE EDITOR.

(2) Fischer, *Ber.*, **34**, 433 (1901).

for glycine ester,[3] it was found advantageous to carry out the poly-condensations with the liquid ester under reduced pressure in order to facilitate the splitting off of the ethanol formed during the poly-condensation.

In these experiments various high alanine peptide esters along with alanine anhydride were obtained. The lowest peptide ester hitherto isolated from the condensation mixtures was the alanine tetrapeptide ethyl ester. This hitherto unknown compound represents the alanine analog of the Curtius biuret base—the glycine tetrapeptide ethyl ester. Like the latter it gives a positive biuret reaction with pink-red color. It is soluble in cold water, ether and other organic solvents in which the corresponding glycine peptide ester does not dissolve. As will become clear later, the solubility of alanine peptide esters is in general greater than that of the corresponding glycine polypeptide esters.

The higher alanine peptide esters obtained from alanine ethyl ester corresponded in average chain length with 10, 14 and 16 units, respectively.

These compounds were isolated by molecular distillation from the reaction mixture, from which the ether soluble part, containing unchanged alanine ethyl ester and the alanine tetrapeptide ethyl ester, was previously removed. During the molecular distillation the alanine anhydride was distilled and the remaining fraction consisted only of the high alanine peptide esters. The separation of the latter had to be effected by this technique as the reaction mixtures were entirely soluble in water and could not be fractionated by differences in the solubility of the various condensation products.

The isolated high alanine peptide ethyl esters are insoluble in ether but soluble in water. Their solutions give a positive ninhydrin reaction and an immediate positive biuret reaction with violet color.

As the carrying out of NH_2-determinations was rendered possible by the solubility of the high alanine peptide esters in water, both the alkoxy- and the amino nitrogen groups were determined. The values for chain length calculated in every case from each of these two independent determinations were identical. Total nitrogen content was also in agreement with the formulas derived from the other data.

The determination of the molecular weight of one representative polypeptide ester, the 10-alanine ethyl ester, by the micro method of Barger[4] showed very satisfactory agreement between the molecular weight found (750) and calculated (756).

Another proof for the structure of the poly-condensation products is furnished by hydrolysis and subsequent quantitative determination of the free alanine formed. The results obtained show that the poly-condensates are built up quantitatively from alanine units linked by —CONH— bonds.

It has been found that the primary polymerization products isolated from the reaction mixtures undergo further poly-condensation at 150°. In this way from 14-alanine ethyl ester, polypeptide esters averaging 17, 19 and 23 units were obtained.

Experimental

Alanine ethyl ester was prepared according to Fischer.[2] Freshly distilled ester was used throughout. For reasons explained in the theoretical part the condensations were carried out in closed vessels at reduced pressure. The temperature was varied between room temperature to about 80°.

Poly-condensation at Room Temperature.—Three grams of freshly distilled alanine ethyl ester was kept in a sealed test-tube under reduced pressure (15 mm.) at room temperature for five months. During standing a precipitate formed which finally filled the whole liquid. After opening the test-tube the mixture gave a strong picric acid test and a positive biuret reaction. The mixture dissolved entirely in water. It was repeatedly treated with ether till the latter gave no positive biuret reaction. The ether solution was in each case separated by centrifuging from the insoluble part and finally the ether extracts combined.

1. Separation of Alanine Tetrapeptide Ethyl Ester from Lower Products.—The ether from the combined extracts was evaporated in a desiccator *in vacuo* at room temperature with rigid exclusion of water, the residue dissolved in 1 ml. of dry ethyl acetate and to the clear solution 2 ml. of petroleum ether was added. The oily precipitate formed was separated from the liquid by decantation, washed with petroleum ether and dried in a desiccator *in vacuo* over sulfuric acid and soda-lime.

The dried semisolid oil (150 mg.) shows positive ninhydrin and biuret reactions, the latter with a pink-red color. On analysis it agreed with alanine tetrapeptide ethyl ester. *Anal.* Calcd. for $C_{14}H_{26}O_5N_4$: N, 16.95; amino N, 4.24; C_2H_5O, 13.64. Found: N, 16.62; amino N, 4.20; C_2H_5O, 13.76.

In order to convert the free tetrapeptide ester into its hydrochloride, it was treated with 2 ml. of absolute ethanol previously saturated with gaseous hydrogen chloride. The residue obtained after drying *in vacuo* was treated once more in the same way. Finally a very hygroscopic semisolid product was obtained which by its chlorine content proved to be alanine tetrapeptide ethyl ester hydro-

(3) Frankel and Katchalski, THIS JOURNAL, **64**, 2264 (1942).

(4) Barger, *J. Chem. Soc.*, **85**, 286 (1904); Barger, *Ber.*, **37**, 1754 (1904).

chloride. *Anal.* Calcd. for $C_{14}H_{26}O_5N_4 \cdot HCl$: Cl, 9.68. Found: Cl, 9.53.

From the solution in petroleum ether only alanine ethyl ester could be isolated.

Alanine tetrapeptide ethyl ester hydrochloride prepared from alanine tetrapeptide synthesized in the usual way was found to be identical with the hydrochloride described above. The free alanine tetrapeptide ethyl ester liberated from this ester hydrochloride was identical with the corresponding product obtained by condensation. It dissolves in ether, alcohol and ethyl acetate. From the latter it is precipitated by addition of petroleum ether.

2. Separation of the Higher Alanine Condensation Products from Alanine Anhydride.—The residue remaining after treatment of the primary reaction mixture with ether dissolved in water, showing a slight alkaline reaction. It gave a strong color reaction with picric acid, indicating the presence of anhydride, and blue-violet biuret reaction, indicating the presence of higher peptide esters. Separation between the anhydride and the higher linear poly-condensation products was carried out by molecular distillation. Alanine anhydride distilled over at an external bath temperature of 140° and a pressure of about 10^{-3} mm.; 1.0 g. of alanine anhydride was obtained; melting point 270°. *Anal.* Calcd. for $C_6H_{10}O_2N_2$: N, 19.70; amino N, 0. Found: N, 19.92; amino N, 0.

The amorphous residue from the molecular distillation was free from alanine anhydride; it gave a negative picric acid test and a blue-violet biuret reaction. It was soluble in water and in 80% alcohol; it showed a distinct alkaline reaction. According to analysis it is 10-alanine ethyl ester. *Anal.* Calcd. for 10-alanine ester: N, 18.52; amino N, 1.85; C_2H_5O, 5.95. Found: N, 18.75; amino N, 1.41; C_2H_5O, 5.67.[5]

The molecular weight of this compound was determined by the micro method of Barger[4] using 80% alcohol as solvent and azobenzene as a standard. By this method it has been determined that the molar concentration of a solution containing 10.5 mg. of 10-alanine ethyl ester in 1.5 ml. of solvent is 0.00933; the molecular weight found from these data is 750, the molecular weight calcd. for the 10-alanine ester $C_{32}H_{56}O_{11}N_{10}$, 756; for the 13-alanine ester, 970.

Poly-condensation at 40°.—Three grams of freshly distilled alanine ethyl ester was allowed to undergo condensation as described above except that the temperature was kept at 40°. The separation of the different fractions was carried out as above. 1.1 g. of alanine anhydride, 120 mg. of alanine tetrapeptide ethyl ester and 200 mg. of high poly-condensation product, which proved on analysis to correspond with 16-alanine ethyl ester, were obtained. *Anal.* Calcd. for $C_{50}H_{86}O_{17}N_{16}$: N, 18.95; amino N, 1.18; C_2H_5O, 3.81. Found: N, 18.90; amino N, 1.32; C_2H_5O, 3.84. In its properties the 16-alanine ethyl ester resembled the 10-alanine ethyl ester.

Poly-condensation at 80°.—Three grams of freshly distilled alanine ethyl ester was kept *in vacuo* at a temperature of 80° for one month and then left for about four months at room temperature. The semi-solid substance thus formed was treated as above: 0.8 g. of alanine anhydride; 100 mg. of alanine tetrapeptide ethyl ester and 300 mg. of the higher poly-condensation product were obtained. The latter is according to analysis 14-alanine ethyl ester. *Anal.* Calcd. for $C_{44}H_{76}O_{15}N_{14}$: N, 18.85; amino N, 1.31; C_2H_5O, 4.32. Found: N, 18.47; amino N, 1.29; C_2H_5O, 4.20. The 14-alanine ethyl ester resembles its linear ester homologs mentioned above.

Quantitative Hydrolysis of 14-Alanine Ethyl Ester.—6.450 mg. of the substance, which according to analysis was 14-alanine peptide ethyl ester, was dissolved in 1 ml. of hydrochloric acid (0.2 ml. of concd. HCl in 0.8 ml. of water) and refluxed for eight hours in a flask with ground-in condenser of 2 ml. content. In order to avoid bumping a few platinum tetrahedra were added.

The determination of the free alanine formed by hydrolysis was carried out according to Friedemann and Kendall.[6] The hydrolyzate was diluted in a 50-ml. flask to 17.5 ml. The flask was kept on a boiling water-bath, and within twenty minutes 3 ml. of sodium nitrite (2.5 g. in 100 ml. of water) was added, and within an additional twenty minutes 3 ml. of urea (7.5 g. in 100 ml. of water). The total solution was brought to 50 ml. and the amount of lactic acid determined according to Friedemann and Kendall using 0.005 *N* iodine solution. *Anal.* Calcd. amount of alanine formed after hydrolysis of 6.45 mg. of 14-alanine ethyl ester: 7.72 mg. Found: 7.20 mg. From the results the conclusion may be drawn that the substance which underwent hydrolysis is built up quantitatively of alanine units held together by —CONH— links. The hydrolysis confirms therefore the other analytical results.

Further Poly-condensation of 14-Alanine Ethyl Ester.—14-Alanine ethyl ester was finely ground and kept at a temperature of about 150° for various periods of time. The progress of condensation was demonstrated by the decrease in alkoxy percentage. No anhydride was found during the operation.

TABLE I

POLY-CONDENSATION OF 14-ALANINE ETHYL ESTER ON KEEPING AT 150°

Time, days	0	5	15	30
C_2H_5O, %	4.20	3.67	3.30	2.66
Calcd. chain length	14	17	19	23

Enzymatic Experiment.—The action of pancreatin was tried on the 16-alanine polymer but the results indicated none or very little enzymatic hydrolysis. These peptides are, however, so far removed from the type of structure to be found in naturally occurring proteins that this failure to observe enzyme action has little bearing on the question of the type of linkage present in the polymer.

Summary

Alanine ethyl ester kept in sealed tubes at reduced pressure and at various temperatures polymerized to compounds whose average size corresponded to 10, 14 and 16 alanine ethyl ester units.

(5) Note by the Editor: These analytical data for nitrogen and amino nitrogen agree better with the formula for a 13-alanine ethyl ester ($C_{41}H_{71}N_{13}O_{14}$). This formula would require N, 18.78 and N in NH_2, 1.44. Similarly, the data for the ethoxy group would agree more closely with an 11-alanine ethyl ester ($C_{35}H_{55}N_{11}O_{12}$) which would require C_2H_5O, 5.44.

(6) Friedemann and Kendall, *J. Biol. Chem.*, **82**, 23 (1929); Friedemann, *J. Inf. Diseases*, **47**, 171 (1930).

14-Alanine ethyl ester underwent further polycondensation at 150° to compounds corresponding to 17, 19 and 23 alanine ethyl ester units.

These alanine peptide esters are, unlike their glycine analogs, soluble in water.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF DUKE UNIVERSITY]

Condensations. XVII. The Acylation of the Anions of Certain Alkyl Esters with Phenyl Esters. A New Method for the Preparation of Ethyl Propionylacetate and Certain Related β-Keto Esters[1,2]

BY B. ABRAMOVITCH AND CHARLES R. HAUSER

The difficulty in effecting satisfactorily the Claisen condensation between two different alkyl esters both of which have α-hydrogen is well known. Even when the ester to be acylated is first converted largely into its anion (or sodium enolate) by means of sodium triphenylmethyl and the anion then treated with the acylating ester, a mixture of β-keto esters is generally obtained.[3] Evidently, the ester anion reacts with the α-hydrogen of the second ester more readily than with its carbonyl group, resulting in a hydrogen exchange to yield a mixture of two different ester anions and two different esters from which four β-keto esters might be formed.

Ester anions are acylated by acid chlorides without first undergoing the hydrogen exchange but with the anion of ethyl acetate (or other ester having two α-hydrogens) the β-keto ester first formed is further acylated by the acid chloride yielding mainly the diacylacetate.[4] Although the latter can be satisfactorily ammonolyzed back to the monoacylacetate the over-all yield is generally low. Thus, when the anion of ethyl acetate was treated with propionyl chloride and the resulting dipropionylacetate ammonolyzed, the over-all yield of ethyl propionylacetate was only 16%.

Obviously a suitable reagent for the direct preparation of monoacylacetates, $RCOCH_2CO_2C_2H_5$, should be one which would acylate the anion of ethyl acetate without first undergoing the hydrogen exchange, but one which would not acylate the monoacylacetate. Such an acylating reagent should presumably have a carbonyl group which is more reactive than that of an alkyl ester but one not as reactive as that of an acid chloride. It seemed possible that phenyl esters (or substituted phenyl esters) might serve as suitable acylating reagents, since, as measured by the rates of alkaline hydrolyses, the carbonyl group of phenyl acetate is approximately thirteen times as reactive as that of ethyl acetate,[5] yet phenyl acetate does not appear to be sufficiently reactive to acylate the anion of ethyl acetoacetate.[6] In agreement with these considerations, treatment of the anion of ethyl acetate with phenyl propionate apparently yielded ethyl propionylacetate,[6] but unfortunately the β-keto ester could not be separated from the phenol which was also produced in the reaction. When *p*-diphenyl propionate was used as propionylating reagent, however, the ethyl propionylacetate was readily separated from the relatively high-boiling by-product, *p*-hydroxydiphenyl. The reactions, including the formation of the ester anion by means of the triphenylmethyl ion, and the conversion of the β-keto ester into its anion, may be represented as follows.

$$CH_3CO_2C_2H_5 + {}^-C(C_6H_5)_3 \longrightarrow (CH_2CO_2C_2H_5)^- + HC(C_6H_5)_3$$

$$C_2H_5CO_2C_6H_4C_6H_5 + (CH_2CO_2C_2H_5)^- \longrightarrow C_2H_5COCH_2CO_2C_2H_5 + {}^-OC_6H_4C_6H_5$$

$$C_2H_5COCH_2CO_2C_2H_5 + (CH_2CO_2C_2H_5)^- \longrightarrow (C_2H_5COCHCO_2C_2H_5)^- + CH_3CO_2C_2H_5$$

Using molecular equivalents of ethyl acetate, sodium triphenylmethyl and *p*-diphenyl propionate, the yield of practically pure ethyl propionylacetate was 44% based on the sodium triphenylmethyl. It can be seen from the equations that although the ethyl acetate first reacts with the

(1) This paper has been constructed from portions of a Thesis presented by B. Abramovitch, in partial fulfillment of the requirements for the Ph.D. degree at Duke University.

(2) This investigation was supported in part by a grant from the Duke University Research Council.

(3) Hudson and Hauser, THIS JOURNAL, **63**, 3158 (1941).

(4) It should be pointed out that the acylation of disubstituted acetic acid esters such as ethyl isobutyrate gives good yields of β-keto esters of the type $RCOC(R'R'')CO_2C_2H_5$ (ref. 3, p. 3159).

(5) Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1940, p. 211.

(6) Unpublished observations by B. E. Hudson in this Laboratory.