139. A Classical Synthesis of the Collagen-like Peptides with the Sequence $Z(GlyProPro)_nOBu^t$ and their Characterization with Circular Dichroism and Ultracentrifugation

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Summary. Oligopeptides with the sequence $Z(GlyProPro)_nOBu^t$ (n = 3–7) were synthesized along a classical pathway. Generally long fragments were condensed in order to allow an easy separation of the products from the reactants. It is established by molecular weight determination and measurements of circular dichroism that the peptides form the collagen-like triple helix in methanol and that they assume the random coil conformation in dilute acetic acid. The circular dichroism spectra agree reasonably well with the corresponding spectra for $(ProProGly)_{10}$ which was obtained by solid phase synthesis in another laboratory.

Introduction. – Polytripeptides with glycine as every third residue and a high content of imino acid residues have the ability to assume the collagen-like triple helical conformation (for recent reviews see [1] and [2]). They serve for a simplified analysis of the structure and of the triple helix $\rightleftarrows$ coil transition of collagen [1] [2] and offer the possibility to study the chain length dependence of the equilibrium and kinetic parameters of this transition. With this information the mechanism and the rate constants of the individual steps in this complex cooperative transition may be evaluated [3] [4].

One of the simplest and most interesting model sequences for collagen is $(GlyProPro)_n$. A polymer with this sequence was first obtained by condensation of the tripeptide ProProGly with tetraethylpyrophosphite [5]. This peptide showed a broad chain length distribution and it was contamined with phosphorus which might interrupt the regular conformation. This polymer was useful for X-ray studies [1] but the desired thermodynamic and kinetic parameters could not be obtained. Oligotripeptides of better defined chain length (n = 5, 10, 15, 20 [6]; n = 10, 12, 14, 15 [7]; n = 7 and 8 [8]) were synthesized by stepwise polymerization of the tripeptide ProProGly using the solid phase method [9]. Although several impressive physicochemical investigations habe been performed with this material [10–16] it may be desirable to have peptide with even higher homogeneity. In the solid phase peptide synthesis several side pro-

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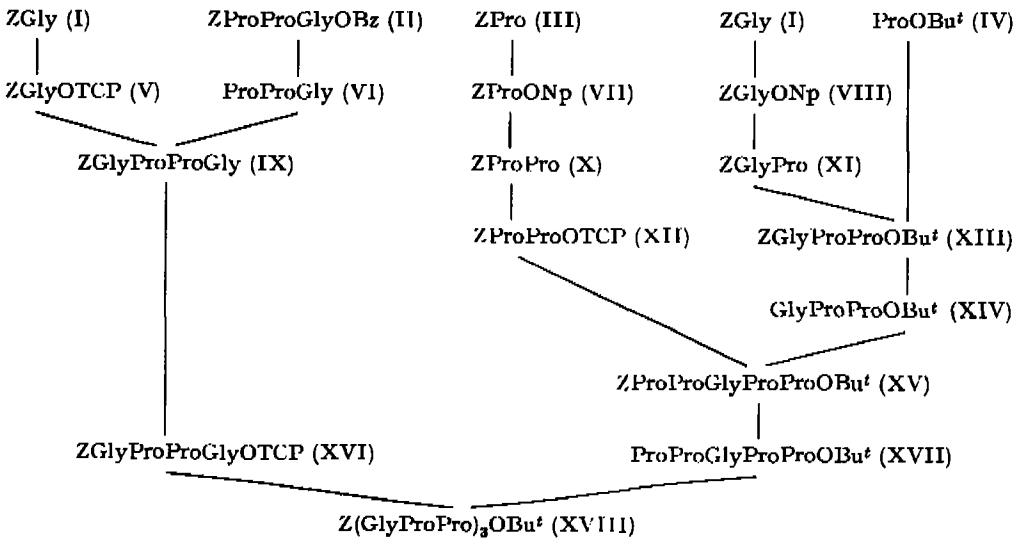
ducts may arise which in general cannot be removed from the main substance [17] [18]. The analysis of the peptide prepared by this method is difficult. Even the high voltage electrophoresis at 4.5–5 kV shows poor resolution at chain lengths higher than 40 amino acid residues [8] [18]. Disturbing irregularities are observed during chain elongation by the solid phase method in the case of $(ProProGly)_n$ [8].

For the reasons discussed we synthesized our peptides along a classical pathway. This made it possible to establish the properties of the intermediate products. The aim was to condense longer fragments in order to facilitate the separation of the products from the reactants by gel chromatography.

Procedure of the Synthesis. – The pathway of the synthesis is given in *Schemes 1–5*:

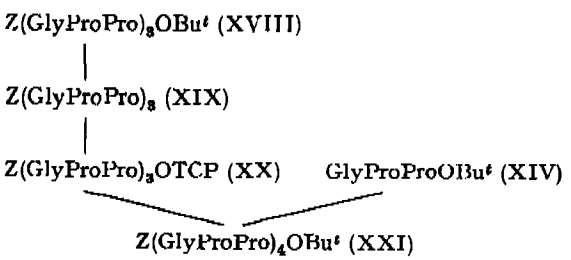
Scheme 1

A) *Z(GlyProPro)$_3$OBu^t*



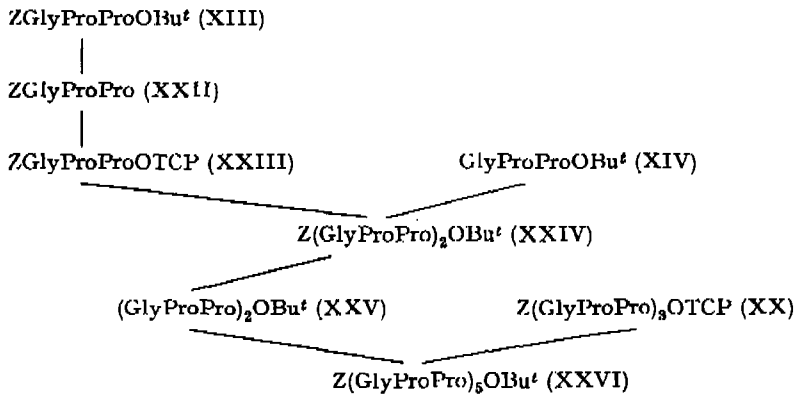
Scheme 2

B) *Z(GlyProPro)$_4$OBu^t*



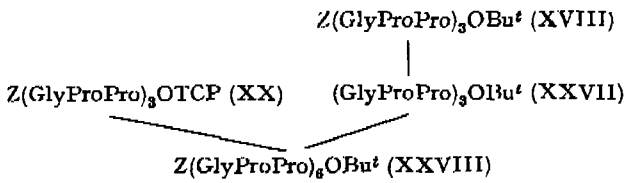
Scheme 3

C) *Z(GlyProPro)$_5$OBu^t*



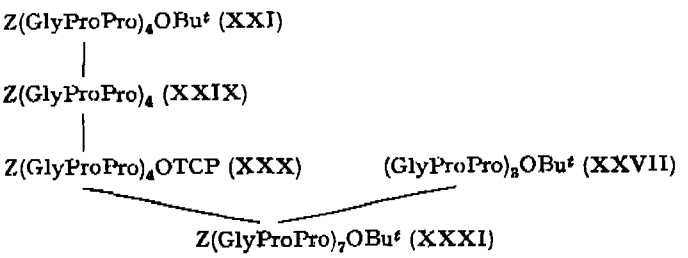
Scheme 4

D) *Z(GlyProPro)$_6$OBu^t*



Scheme 5

E) *Z(GlyProPro)$_7$OBu^t*



The abbreviations are:
OBz = benzyl ester; OBu^t = *t*-butyl ester;
Z = benzyloxycarbonyl; OTCP = 2,4,5-trichlorophenyl ester;
ONp = *p*-nitrophenyl ester.

The tripeptide ZProProGlyOBz (II) was obtained according to *Wünsch et al.* [19] with use of the carbodiimid method. They also prepared the free tripeptide (VI) by catalytic hydrogenation. This free peptide is very easily decomposed to the diketopiperazine of two proline residues and glycine at warming or at standing at room temperature. This undesired reaction may totaly be avoided if the hydrogenation is carried out in glacial acetic acid. Not even traces of glycine could be detected in the product by electrophoresis on a silicagel plate. One equivalent of acetic acid could not be removed from the substance by keeping over solid KOH *in vacuo* overnight. The substance decomposes only after melting. If an aqueous solution of this peptide is rendered alkaline decomposition starts. Therefore one finds in the product of the next reaction step traces of $ZGly_2$. This can almost be removed by precipitating the peptide (IX) several times.

All 2,4,5-trichlorophenylesters were prepared according to *Pless et al.* [20] by the method of *Rothe et al.* [21]. Because of the tendency of derivatives activated at the carboxyl group of ZGlyPro to cyclise [22], synthesis of ZGlyProProOBu^t (XIII) was effected *via* activation of the imino group of ProOBu^t (IV) by the phosphorazo method [23–25]. The coupling of active esters with peptides of amino acids was carried out following the procedure of the synthesis of oligoprolines by *Rothe et al.* [26].

Experimental Part

The thin layer chromatography (TLC.) was performed on silicagel plates (0.25 mm layer) (*Merck*, Darmstadt) with two solvent systems: A) *n*-butanol/acetic acid/water 3:1:1 (*v/v*) B) *n*-butanol/pyridine/acetic acid/water 60:20:6:24 (*v/v*). The corresponding Rf-values are designated Rf_A and Rf_B. All hydrogenations were performed under normal pressure at a slow hydrogen flow in the presence of Pd-Black (purchased by *Fluka AG*, chem. Fabrik, Buchs SG, Switzerland) for a period of 10 h if not stated otherwise.

Molecular weights were determined by the *Yphantis* midpoint equilibrium sedimentation method at 30000 rpm [27]. A model E analytical ultracentrifuge (*Beckman* Instruments) was applied. The partial specific columnes were determined with a digital densitometer (*A. Paar*, Austria) according to *Kratky et al.* [28]. The circular dichroism measurements were performed with a *Cary* 61 spectropolarimeter (*Varian*) in thermostated cells of 0.1 to 1 mm pathlength (*Opticell* and *Perkin Elmer*). The methanol used for CD.-measurements had spectral grade (*E. Merck*, Darmstadt). Proline was purchased from *Fluka AG*, Buchs SG, Switzerland, $[\alpha]_D = -85.5°$ ($c = 5$, H_2O). Glycine had analytical grade (*E. Merck*, Darmstadt).

The peptides $(ProProGly)_5$ and $(ProProGly)_{10}$ prepared by the solid phase method were products from *Protein Research Foundation*, Minoh, Osaka, Japan.

A) Synthesis of Z(GlyProPro)$_3$OBu^t (XVIII). – *ProProGly · CH_3COOH (VI).* 37.8 g of ZProProGlyOBz (II) [19] ($[\alpha]_D = -115.1°$; m.p.: 120–122°), were dissolved in 400 ml of glacial acetic acid and hydrogenated. After completion of the reaction the catalyst was filtered off and the solvent was evaporated under vacuum at 20°. An oil remained which cristallized upon stirring with ether. The chromatographically pure substance was filtered, washed with ether and dried over KOH *in vacuo* (0.1 mm Hg) overnight. The yield was 25.2 g (100%) $[\alpha]_D = -82.4°$; $[\alpha]_{546} = -97.7°$ ($c = 1.233$, CH_3OH), m.p.: 123–124° (dec.). The substance is found to be pure on electrophoresis on a kieselgur/silicagel 2:1 plate at 1 kV [8].

ZGlyProProGly (IX). 22.3 g of ZGlyOTCP (V) [20, 21] (m.p.: 107–108°), 22.6 g of ProProGly (VI) and 20 ml of freshly distilled triethylamine were dissolved in 250 ml of dry ethyl acetate and stirred at room temp. for 24 h. Afterwards, the reaction mixture was extracted with sat. $KHCO_3$-solution. The aqueous layer was acidified with conc. hydrochloric acid cautiously to pH 2 and extracted with 3 portions of chloroform. The combined organic extract was washed with water and dried over Na_2SO_4 overnight. The chloroform was evaporated under vacuum. A white amorph-

ous powder remained which contained traces of ZGly and ZGlyGly. These can be removed by repeated precipitation from chloroform/petroleum ether. The substance weakens at 80°. $[\alpha]_D = -124.1°$ ($c = 1$, CH_3OH); yield: 20 g (76%); $Rf_A = 0.41$, $Rf_B = 0.31$.

ZGlyPro (*XI*). 80 g of proline ($[\alpha]_D = -85.5°$) was dissolved in a mixture of 400 ml water and 400 ml pyridine and the pH was raised to 9.4 with 4N NaOH. A solution of 153 g of ZGlyONp (VIII) [29] [21] (m.p.: 127–128°) in 200 ml of dry pyridine was dropped into the reaction mixture slowly. The pH was maintained at 9.4 with 4N NaOH overnight by means of a pH state. The pH was then lowered to 8.0 by the addition of solid citric acid and the yellow solution was extracted with diethylether in a *Kutscher-Steudel*-apparatus for 24 h. The pale yellow aqueous layer was saturated with NaCl and acidified cautiously with conc. hydrochloric acid to pH 1.5. The product precipitated, was filtered off and washed with water. The yield of the crude product was 129 g (91%). This was recrystallized from ethyl acetate. $[\alpha]_D = -75°$ ($c = 1$, CH_3OH); m.p.: 150–152°; yield: 123 g (87%), $Rf_A = 0.54$, $Rf_B = 0.49$.

ZGlyProProOBu^t (*XIII*). A solution of 28 g of ProOBu^t (IV) [30] ($[\alpha]_D = -32.8°$ ($c = 1$, OH_3OH); b.p.: 113–114°/11 Torr) in 300 ml of dry pyridine was cooled to −15° and 14.3 ml of PCl_3 were added dropwise. The temperature was not allowed to rise over 0°. The cooling bath was taken off and the arising suspension was stirred for 30 min. After addition of 50 g of ZGlyPro (XI) the reaction mixture was heated to 70° for 4 h, cooled to room temp. and filtered. The brown filtrate gave an oil upon evaporation, was dissolved in 300 ml chloroform and extracted with 1N hydrochloric acid, 2N Na_2CO_3 and with water successively. After drying over Na_2SO_4 and evaporation an oil was yielded which crystallizes upon stirring with petroleum ether (b.p.: 40–60°). The product was recrystallized from ethyl acetate. Yield: 52.5 g (70%). $[\alpha]_D = -147.5°$ ($c = 1$, CH_3OH); m.p. 111–113°; $Rf_A = 0.70$, $Rf_B = 0.70$. Further physical data are listed in Table 1.

GlyProProOBu^t · HCl (*XIV*). 25.5 g of ZGlyProProOBu^t (XIII) were dissolved in 200 ml of methanol and hydrogenated in the presence of Pd-black as catalyst. The pH was maintained at 4.0 by adding of a saturated solution of dry HCl-gas in methanol with a pH state. The end of the reaction was determined chromatographically. The solution was filtered and evaporated. The product was chromatographically pure. The yield was 20.0 g (100%). $[\alpha]_D = -138.2°$; m.p.: 126–129°; $Rf_A = 0.48$, $Rf_B = 0.58$. The mass spectrum was consistent with the expected structure (free peptide ester). Sometimes, especially when the reaction mixture was kept at lower pH, the carboxyl blocking group was also cleaved off. Therefore, we later hydrogenated the peptide in glacial acetic acid. This method yielded an oil which had the same chromatographical properties as the hydrochloride but we never got partial cleaving of the ester group.

ZProPro (*X*). The synthesis of ZProPro was completely analogous to the preparation of ZGlyPro (XI). The product was recrystallized from methanol. The yield was 94%. $[\alpha]_D = -82.5°$ ($c = 1$, DMF); m.p. 191.5–193.5°; $Rf_A = 0.57$, $Rf_B = 0.49$.

ZProProOTCP (*XII*). 17.7 g ZProPro (X) and 11.1 g of 2,4,5-trichlorophenol were dissolved in 100 ml of pyridine and diluted with 100 ml of chloroform. 11.6 g of dicyclohexylcarbodiimide (DCCI) were dissolved in 200 ml of abs. chloroform and added dropwise to the reaction mixture at room temperature. The arising suspension was stirred overnight. The dicyclohexylurea was filtered off (10.3 g (81.5%)) and the filtrate was evaporated *in vacuo* to an oil which solidified upon vigorous stirring with petroleum ether (b.p.: 40–60°) after 45 min. Recrystallized from ethylacetate/petroleum ether the product was a white powder. Yield: 25.0 g (93%), m.p.: 101–103°; $[\alpha]_D = -106.2°$ ($c = 1$, CH_3OH); $Rf_A = 0.73$, $Rf_B = 0.68$.

ZProProGlyProProOBu^t (*XV*). 19.0 g of GlyProProOBu^t · HCl (XIV) were dissolved in 110 ml of water and diluted with 110 ml of pyridine. A solution of 23.0 g of ZProProOTCP (XII) in dry pyridine was added dropwise to the reaction mixture. The pH was maintained at 9.1 with 4N NaOH overnight. The arising clear solution was acidified with conc. hydrochloric acid to pH 5 and afterwards with solid citric acid to pH 3. The solution became turbid and was extracted with 500 ml of chloroform. The organic layer was separated and extracted successively with 100 ml of 2N citric acid, two times with 100 ml of 2N Na_2CO_3-solution and finally with three times 150 ml of water. Drying over Na_2SO_4 and evaporation yielded 25.1 g of an amorphous, white powder (75%). $[\alpha]_D = -182.5°$ ($c = 1$, CH_3OH), $Rf_A = 0.48$, $Rf_B = 0.68$. The Mol.-Wgt. was found to be 635 (equilibrium sedimentation in DMF and 650 (gel chromatography with Sephadex LH 20 in $(CH_3)_2SO$). The calculated value is 654. A small portion of the substance was treated with

anhydrous trifluoroacetic acid. The mass spectrum of it was found to be consistent with the structure of the free carbobenzoxypentapeptide. The amino acid analysis gave a ratio of glycine to proline 1:3.98 (calc.: 1:4).

ProProGlyProProOBu^t · CH$_3$COOH (XVII). 21.5 g of ZProProGlyProProOBu^t (XV) were hydrogenated in 250 ml of glacial acetic acid for 10 h in the presence of Pd-black. After evaporation a chromatographically pure oil remained which was used without further characterisation; $Rf_A = 0.36$, $Rf_B = 0.35$.

ZGlyProProGlyOTCP (*XVI*). The preparation of ZGlyProProGlyOTCP was performed analogously to that of ZProProOTCP (XII). The substance was a yellowish powder with a point of weakening at 64°. $[\alpha]_D = -115.8°$ ($c = 1$, CH_3OH); $Rf_A = 0{,}68$, $Rf_B = 0.76$. Yield: 98%.

Z(GlyProPro)$_3$OBu^t (*XVIII*). 18.6 g of ProProGlyProProOBu^t · CH$_3$COOH (XVII) were emulgated in 90 ml of pyridine and diluted with 90 ml of water. The mixture was cooled to 0°, set slowly to pH 9.3 with 4N NaOH and warmed up to room temperature. A suspension of 18.7 g of ZGlyProProGlyOTCP (XVI) in 60 ml of dry pyridine was dropped into the stirred reaction mixture. The pH was maintained at 9.3 with 4N NaOH in a pH state. The reaction was allowed to proceed for 24 h. The turbid solution was cooled then to 0° and acidified with solid citric acid to pH 3 and extracted with 2 times 600 ml of chloroform. The organic layer was extracted with saturated Na_2CO_3-solution and twice with water. After drying and evaporation the remaining foam was chromatographed over Sephadex LH 20. The eluant was methanol. The first fraction appeared to be chromatographically pure: $Rf_A = 0.38$, $Rf_B = 0.48$, $[\alpha]_D = -224.2°$ ($c = 0.82$, CH_3OH), yield: 15.2 g (54%). Further physical properties are listed in Table 1.

B) Preparation of Z(GlyProPro)$_4$OBu^t – *Z(GlyProPro)$_3$* (*XIX*). 1 g of Z(GlyProPro)$_3$-OBu^t (XVIII) was dissolved in 10 ml of anhydrous trifluoroacetic acid and stirred at room temp. for 15 min. The solvent was evaporated *in vacuo* and stirred with three portions of ether. The material was a white, amorphous powder which weakens at 130°; $Rf_A = 0.25$, $Rf_B = 0.22$, $[\alpha]_D = -211.2°$ ($c = 1.4$, CH_3OH). The yield was 940 mg (100%).

Z(GlyProPro)$_3$OTCP (*XXI*). 600 mg of Z(GlyProPro)$_3$ (XIX) were dissolved in 10 ml of pyridine. 230 mg of 2,4,5-trichlorophenol and 250 mg of DCCI were added. This solution was stirred overnight, the precipitated dicyclohexylurea was filtered off and the solvent was evaporated. The residue was taken up in 250 ml of chloroform and successively extracted with 1N HCl, 2N Na_2CO_3-solution and with water. The organic phase was dried over Na_2SO_4 and evaporated. An amorphous, faintly brownish foam remained which was chromatographically pure: $Rf_A = 0.36$, $Rf_B = 0.55$, $[\alpha]_D = -162.5°$ ($c = 1$, CH_3OH); yield: 500 mg (70%).

Z(GlyProPro)$_4$OBu^t (*XXI*). 460 mg of GlyProProOBu^t · HAc (XIV) were dissolved in 10 ml of pyridine/water 1:1 (*v/v*). The pH was adjusted to 9.3 with 4N NaOH. 650 mg of Z(GlyProPro)$_3$-OTCP (XXI) dissolved in 3 ml of pyridine were added dropwise over a period of 1/4 h. The pH was maintained at 9.3 with 4N NaOH by means of a pH-state and the solution was stirred overnight. The pH was the lowered to pH 7 with solid citric acid and the solvent was evaporated *in vacuo*. The residue was taken up in 100 ml of chloroform and successively extracted with twice 25 ml of a solution of 5% citric acid and 5% NaCl (*w/v*), twice 25 ml of a mixture of 1N NaOH and saturated NaCl-solution 1:1 and three times with 25 ml of saturated NaCl-solution. The organic layer was dried over Na_2SO_4 and evaporated. The crude product (650 mg; yield: 89%) was fractionated over a Sephadex LH 20 column with methanol as solvent. The first fraction gave a chromatographically pure, white, amorphous powder. Yield: 490 mg (67%); $Rf_A = 0.23$, $Rf_B = 0.40$. Further physical properties are listed in Table 1.

C) Preparation of Z(GlyProPro)$_5$OBu^t – *ZGlyProPro* (*XXII*). 5 g of ZGlyProProOBu^t were dissolved in 50 ml of freshly distilled anhydrous trifluoroacetic acid and stirred for 15 min. The solution was then evaporated to dryness and the remaining oil was triturated with ether until the odor of trifluoroacetic acid has disappeared. The residue was taken up into 100 ml of chloroform and extracted with 25 ml 0.5N citric acid. The organic layer was dried over Na_2SO_4 and evaporated. A white amorphous powder appeared which was chromatographically pure; $Rf_A = 0.51$, $Rf_B = 0.51$. Yield: 2.4 g (54%).

ZGlyProProOTCP (*XXIII*). 2.4 g of ZGlyProPro (XXII) and 1.29 g of 2,4,5-trichlorophenol were dissolved in 30 ml of pyridine. To this solution 1.59 g of DCCI were added and the reaction

mixture was stirred overnight. The precipitate was filtered off, the remaining solution evaporated, dissolved in chloroform and extracted with 1N hydrochloric acid, 2N Na_2CO_3 and water. The organic phase was dried over Na_2SO_4 and evaporated. After trituration with petroleum ether a faintly brown solid material arose, which was chromatographically pure. Yield: 2.1 g (60%); $Rf_A = 0.78$, $Rf_B = 0.81$.

Z(GlyProPro)$_3$OBu^t (XXIV). Starting from 2.1 g of ZGlyProProOTCP and 2.8 g of GlyProProOBu^t the synthesis was performed analogously to the synthesis of Z(GlyProPro)$_4$OBu^t (XXI). The yield was 850 mg (34%); $Rf_A = 0.52$, $Rf_B = 0.57$. The mass spectrum is consistent with the assumed structure. Further data are listed in Table 1.

(GlyProPro)$_3$OBu^t (XXV). 500 mg of Z(GlyProPro)$_3$OBu^t were hydrogenated in glacial acetic acid as described above. The chromatographically pure product was used without further characterization.

Z(GlyProPro)$_5$OBu^t (XXVI). Starting from 500 mg of Z(GlyProPro)$_2$OTCP and 450 mg of GlyProProOBu^t · HAC the peptide was synthesized analogously to the procedure described for Z(GlyProPro)$_4$OBu^t. The yield was 115 mg (17%); $Rf_A = 0.06$, $Rf_B = 0.31$. Further data are given in Table 1.

D) Preparation of Z(GlyProPro)$_6$OBu^t(XXVIII). – *(GlyProPro)$_3$OBu^t · CH$_3$COOH (XX)*. 770 mg of Z(GlyProPro)$_3$OBu^t (XVIII) were hydrogenated in 10 ml of glacial acetic acid. The oil arising after evaporation solidified upon addition of ether. The product was chromatographically pure; $Rf_A = 0.20$, $Rf_B = 0.38$. Yield: 710 mg (100%).

Z(GlyProPro)$_6$OBu^t (XXII). The synthesis of the octadecapeptide was carried out analogously to that of the nonapeptide (XVIII). It was eluted from the Sephadex LH 20 column as the first, well separated fraction and was chromatographically pure; $Rf_A = 0.04$, $Rf_B = 0.25$; m.p.: 192–193°. Yield: 24%.

E) Preparation of Z(GlyProPro)$_7$OBu^t – *Z(GlyProPro)$_4$ (XXIX)*. Starting from 460 mg of Z(GlyProPro)$_4$OBu^t the peptide was prepared analogously to the procedure described for Z(GlyProPro)$_3$. A very small spot on the starting point of the chromatogramm was observed indicating that traces of the free peptide arose. The yield was 391 mg (90%); $Rf_A = 0.13$, $Rf_B = 0.21$.

Z(GlyProPro)$_4$OTCP (XXX). 120 mg of (Z(GlyProPro)$_4$ (XXIX) were suspended in 0.5 ml of $(CH_3)_2SO$. The substance was dissolved upon the addition of 1.5 ml of dry pyridine. 30 mg of 2,4,5-trichlorophenol and 50 mg of DCCI were added. The reaction mixture was stirred overnight. After filtration of the dicyclohexylurea and evaporation of the solvent a brownish, amorphous powder was obtained upon trituration with ether. The yield was 91 mg.

Z(GlyProPro)$_7$OBu^t (XXXI). The procedure of the preparation of Z(GlyProPro)$_7$OBu^t was analogous to that of Z(GlyProPro)$_3$OBu^t, starting from 362 mg Z(GlyProPro)$_4$OTCP (XXX) and 290 mg of (GlyProPro)$_3$OBu^t (XXVII). 31 mg of solid material were yielded; $Rf_A = 0.02$, $Rf_B = 0.20$.

Discussion of the molecular homogenity of the peptides. – The peptides are assumed to be pure and homogeneous by chain length for the following reasons: 1) All intermediates are pure according to TLC. in the two solvent systems discussed; 2) In order to characterize the shorter peptides mass spectra, determination of the molecular weight by ultracentrifugation or amino acid analysis were performed. All determined values are consistent with the calculated ones or the assumed structure; 3) The homogeneity of the peptides with $n > 3$ can be proved by TLC. in the solvent system B (see Fig. 1). No side products can be detected; 4) All peptides appear as a well separated sharp peak when chromatographed on a Sephadex LH 20 column with methanol as eluant. The side bands can be identified as the reactants by TLC. (see Fig. 2). Peptides of the sequence Z(GlyProPro)$_n$OMe have been prepared by a Russian group [31]. Unfortunately only few analytical data are available for direct comparison

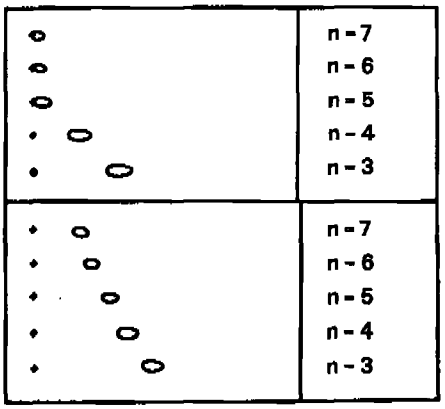


Fig. 1. *Thin layer chromatogramm of Z(GlyProPro)$_n$OBu^t on silica gel in the solvent systems A and B.* The detection of the spots was performed successively with iodine vapours (positive), ninhydrine (negative) and with Cl_2/*o*-tolidine (positive)

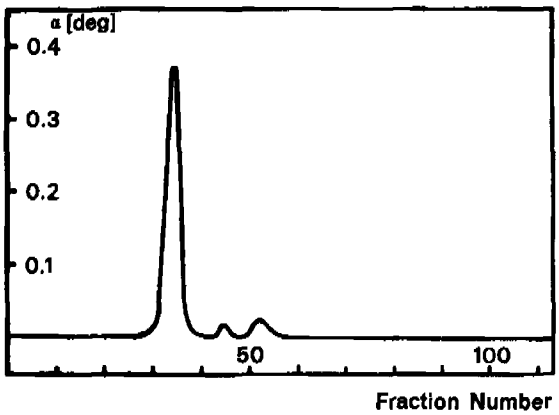


Fig. 2. *Gel chromatogramm of Z(GlyProPro)$_6$OBu^t as detected by the optical rotation of the eluant.* The first peak corresponds to Z(GlyProPro)$_6$OBu^t, the second to Z(GlyProPro)$_3$OTCP and the third to (GlyProPro)$_3$OBu^t as shown by TLC

with our peptides. They were prepared by stepwise elongation with a GlyProPro tripeptide unit.

Further characterization of the peptides Z(GlyProPro)$_n$OBu^t (n = 4–7). – According to sedimentation equilibrium the peptides if dissolved in aqueous acetic acid (1% acetic acid for n = 4,5 and 6 and 50% acetic acid for n = 7) have a molecular weight which corresponds almost to the calculated values for the single chains (see Table 1). The partial specific volume was found to be 0.77 cm^3/g at 20° for a solution of Z(GlyProPro)$_3$OBu^t. This value was assumed to be the same for the other peptides. In methanol, however, molecular weights are found which are essentially three times the molecular weights of the monomeric peptide chains (see Table 1). They also correspond considerably well with the values calculated for the triple helical peptides. The partial specific volume was determined as 0.82 cm^3/g (again only for Z(GlyProPro)$_3$ OBu^t).

Table 1

Peptide	Solvent	$\bar{v}$ [ml/g]*	Calc. Mol.-Wgt. trimer	Calc. Mol.-Wgt. monomer	$\bar{M}_w$* (ultra-centrifuge)	$[\theta]_{225}$* deg · cm²/ decimol	$[\theta]_{200}$ deg · cm²/ decimol
n = 1	DMF	0.82		459	455		
n = 2	Methanol	0.816	2130	710	720		
n = 2	DMF	0.815	2130	710	720		
n = 3	Water	0.77	2883	961	950		
n = 3	Methanol	0.816	2883	961	980		
n = 4	1% AcOH	0.77[a])	3636	1212	1300	– 300	–29000
n = 4	Methanol	0.816[a])	3636	1212	3200	700	–24500
n = 5	1% AcOH	0.77[a])	4389	1463	1650	– 400	–29500
n = 5	Methanol	0.816[a])	4389	1463	4600	4500	–30000
n = 6	1% AcOH	0.77[a])	5142	1714	1850	– 500	–31000
n = 6	Methanol	0.816[a])	5142	1714	5100	6500	–33000
n = 7	50% AcOH	0.77[a])	5895	1965	2600	200[b])	–31500[b])
n = 7	Methanol	0.816[a])	5895	1965	6850	7000	–36000
n = 10 free	Methanol					5000	–31000
peptide	1% AcOH at 50°					–1500	–23000

[a]) Measured for n = 3.
[b]) Values determined in 1% acetic acid.

* $\bar{v}$ = partial specific volume
$\bar{M}_w$ = weight average molecular weight
$[\theta]_{225}$ = molar ellipticity per amino acid residue at 225 nm

Further support for the formation of the triple helical conformation in methanol comes from the circular dichroism spectra (see Fig. 3). In this solvent spectra are found which are typical for the triple helical conformation [32] [33]. They agree roughly with the corresponding spectrum of the peptide $(ProProGly)_{10}$ synthesized on a polymer resin [6]. In dilute acetic acid at 20° and a peptide concentration of 0.5 to 1 mM the positive peak at 225 nm has disappeared and the spectra now correspond to denaturated collagen [31] (Fig. 4). Again reasonable agreement is observed with the spectrum of coiled $(ProProGly)_{10}$ in the same solvent at 50°. The higher temperature was chosen because at 20° this peptide is helical in 1% acetic acid because of its larger chain length. The small through near 230 nm which was reported for $(ProProGly)_{10}$ previously [2] could not be reproduced in the present study.

Collagen has a very negative minimum in its CD.-spectrum at 200 nm which is lowered by about 80% upon heat denaturation. The helix coil transition of our peptides is accompanied by smaller changes in this spectral region and the magnitude and direction of the effect is chain length dependent. For n = 7 the usual decrease in negative ellipticity is found upon coil formation. At n = 5 and 6 almost no change is observed and at n = 4 the effect is reversed (see Table 1 and Fig. 3 and 4). This chain

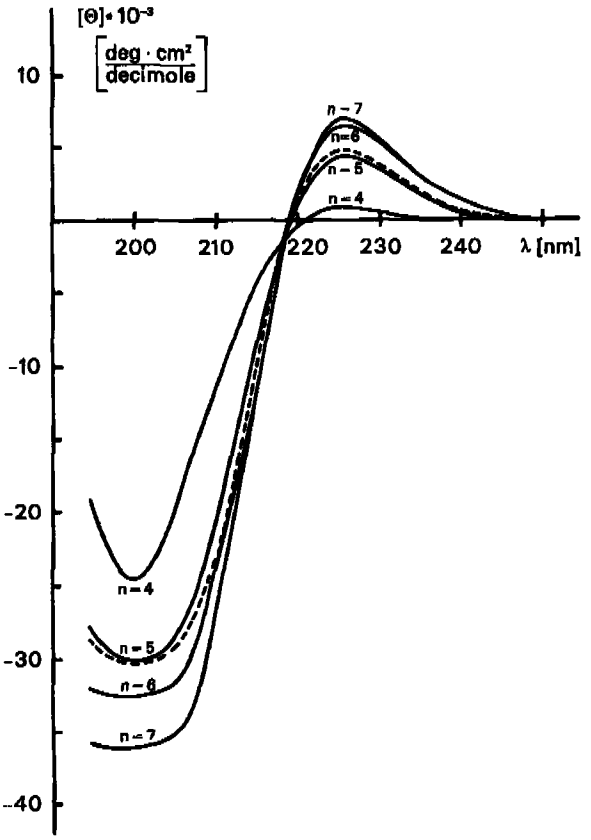


Fig. 3. *CD. spectra of the peptides Z(GlyProPro)$_n$OBu^t in methanol at 20°.* Concentration: 1 mg/ml. For comparison the spectrum of (ProProGly)$_{10}$ in methanol is also given (broken line)

length dependence may result from long range contributions of the coupling of peptide chromophore along the chain. It has to be kept in mind, however, that the unknown contribution of the end groups is more important for shorter than for larger chain lengths. The small positive peak at 225 nm which is observed only in the helical state gradually increases with chain length. This peak serves as the best signal for following the formation of the triple helix. The minimum which shows up at 200 nm in the coiled state exhibits nearly no chain length dependence. Its value is much higher than for collagen, a fact which may be explained by the restriction of rotational freedom imposed by the larger fraction of ProPro pairs in the peptides.

The helix $\rightleftarrows$ coil transition of the peptides Z(GlyProPro)$_n$OBu^t may be induced by a change of concentration, temperature and the solvent composition. A study and analysis of the equilibrium and the kinetics of this transition and a comparison of that of other collagen like peptides will be reported in a successive publication.

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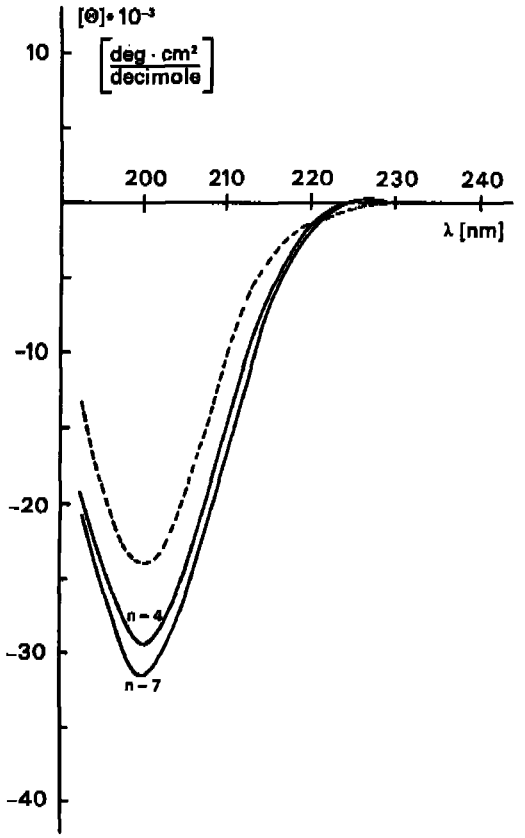

Fig. 4. *CD. spectra of the peptides Z(GlyProPro)$_n$OBu^t in 1% acetic acid at 20°.* Concentration: 1 mg/ml. The dashed line corresponds to the spectrum of (ProProGly)$_{10}$ in 1% acetic acid at 50°

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140. Synthesis of 4-Oxo-1,2,3,4-tetrahydropyridine (2,3-Dihydro-4(1*H*)pyridinone)

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(7. V. 75)

Summary. N-Ethoxycarbonyl-4-pyridone is reduced to N-ethoxycarbonyl-4-oxo-1,2,3,4-tetrahydropyridine by sodium borohydride in *t*-butyl alcohol containing ethyl chloroformate. Saponification of the product leads to 4-Oxo-1,2,3,4-tetrahydropyridine.

1. Introduction. – Some N-alkylated 4-Oxo-1,2,3,4-tetrahydropyridines have been most successfully synthesized by reduction of the corresponding N-alkyl pyridones with hydroaluminates. A first example was given by *Winterfeldt* [1] who reduced N-[2-(indol-3-yl)-ethyl]-4-pyridone with lithium aluminium hydride to the corresponding N-alkyl-4-oxo-1,2,3,4-tetrahydropyridine. Other N-alkyl-4-oxo-1,2,3,4-tetrahydropyridines were prepared by *Tamura et al.* [2] treating N-alkyl pyridones with lithium triethoxy aluminium hydride. Such compounds have also been obtained by other procedures: An example by *Hebky et al.* [3] showed that they are accessible by catalytic hydrogenation of pyridones; *Stütz et al.* [4] found an opposite mode of access: dehydrogenation of an N-alkyl piperidone occurred following a peracid and acetic anhydride/base treatment by means of a modified type of the *Polonovsky* reaction. A total synthetic route to some 2,6-disubstituted 4-oxo-1,2,3,4-tetrahydropyridines is reported by *Sugiyamas* group [5] condensing *Schiff*-bases with dialkali salts of β-diketones.