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The Synthesis of a Series of *N*-*t*-Amyloxycarbonyl-oligo-L-proline

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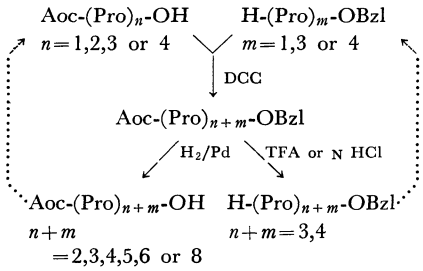
A series of *N*-protected L-proline oligomers, the di-, tri-, tetra-, penta-, hexa- and octamer, were synthesized by conventional methods. The proline imino-group was protected by the *t*-amyloxycarbonyl (Aoc) group, and the carboxyl group was protected as the benzyl ester during the synthesis. These Aoc-oligoprolines were valuable in elucidating the relationship between the conformation and the degree of polymerization of the polyprolines. The Aoc-protecting group was useful in purifying the intermediates and in obtaining crystals of the final compounds.

This paper will report the synthesis of a series of proline oligomers, which have been used in elucidating the relationship between the number of proline residues and the steric structure of the molecules.[1,2] Since a prolyl-prolyl linkage is susceptible to aqueous caustic alkali, the saponification of the ester group was avoided in the synthesis. Generally, the *t*-amyloxycarbonyl (Aoc) group[3] was used to protect an imino group of oligoprolines and the carboxyl groups were protected as benzyl esters. The Aoc-group is known to be stable under the conditions used for the catalytic hydrogenolysis of benzyl esters, and it can be removed by treatment with N hydrogen chloride in an organic solvent or by treatment with trifluoroacetic acid at room temperature for 30 min, the benzyl ester group being unaffected by this treatment.[3] Therefore, each of the protecting group can be removed independently. Furthermore, the use of these lipophilic protecting groups was advantageous in increasing the solubility of the proline oligomers in usual organic solvents and in obtaining many of them as nice crystals. The proline peptides were coupled by the dicyclohexylcarbodiimide (DCC) method, because there is no racemization problem in the activation of proline residues. Since Aoc-derivatives are very soluble in usual organic solvents, as has been mentioned above, urea or ureide compounds could be easily removed from the reaction mixture. Thus, a series of Aoc-oligoprolines were synthesized, as is shown in the following scheme:



The physical constants and solubilities of the compounds are summarized in Tables 1 and 2.

The molecular rotations of the respective compounds ($n+m$=2—8) seem to conform to the additive rule in this region, as is shown in Fig. 1.

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1) T. Isemura, H. Okabayashi and S. Sakakibara, *Biopolymers*, **6**, 307 (1968).

2) H. Okabayashi, T. Isemura and S. Sakakibara, *ibid.*, **6**, 323 (1968).

3) S. Sakakibara, M. Shin, M. Fujino, Y. Shimonishi, S. Inouye and N. Inukai, This Bulletin, **38**, 1522 (1965).

Table 1. Physical constants and solubilities of Aoc-oligoproline benzyl esters

Aoc-(Pro)$_{n+m}$-OBzl $n+m$	Mp °C	Optical rotation			Solubility		
		$[\alpha]_D$ in ethanol	c	Temp. °C	acetone	ethyl acetate	ethanol
1+1 (III)	oil						
2+1 (V)	93.5—94.5	−171°	2.2	25	soluble	soluble	soluble
3+1 (VII)	101.0—103.0	−211°	2.3	24	soluble	soluble	soluble
2+3 (IX-a)	195.5—197.5	−251°	2.0	24	soluble in hot.	soluble in hot.	soluble
4+1 (IX-b)	197.0—198.5	−254°	2.0	24	soluble in hot.	soluble in hot.	soluble
3+3 (XI)	228.5—230.5	−270°	2.1	24	slightly soluble in hot.	slightly soluble in hot.	soluble
4+4 (XIII)	261.0—262.5	−309°	1.2	22	insoluble	insoluble	soluble

Table 2. Physical constants and solubilities of Aoc-oligoprolines

Aoc-(Pro)$_{n+m}$-OH $n+m$	Mp °C	Optical rotation			Solubility		
		$[\alpha]_D$ in ethanol	c	Temp. °C	acetone	ethyl acetate	ethanol
1+1 (IV)[a]	157.0—159.0	−113°	2.0	25	soluble	soluble	soluble
2+1 (VI)	200.0—201.5	−170°	2.2	25	soluble	slightly soluble in hot.	soluble
3+1 (VIII)	207.5—208.5	−227°	2.1	24	soluble in hot.	insoluble	soluble
2+3 (X-a)	224.5—226.0	−248°	2.3	24	slightly soluble in hot.	insoluble	soluble
4+1 (X-b)	224.5—226.0	−248°	2.3	24	slightly soluble in hot.	insoluble	soluble
3+3 (XII)	240.0—242.0	−291°	2.1	24	insoluble	insoluble	soluble
4+4 (XIV)	280.0	−318°	0.9	22	insoluble	insoluble	soluble in hot.

a) Reported in Ref. 5: mp 155—158°C, $[\alpha]_D^{21}$ −112.8° (c 1, ethanol).

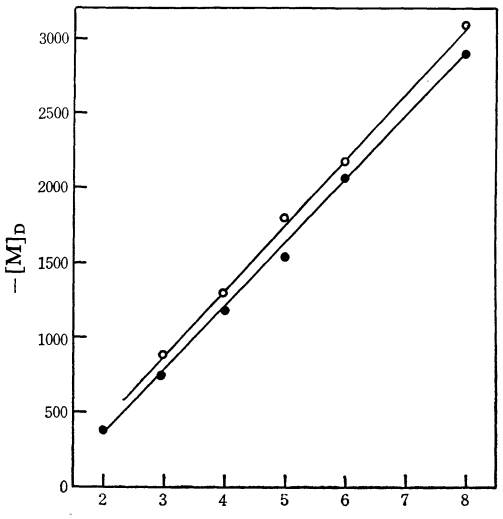

Fig. 1. Molecular rotation change of Aoc-oligo-L-prolines and their benzyl esters.

$$[M]_D = \frac{[\alpha]_D \times (\text{mol wt})}{100}$$

○ Aoc-oligo-L-proline benzyl esters,
● Aoc-oligo-L-proline

4) The separated spots were located by a ninhydrin reaction at 100°C.

Experimental

The physical constants and solubilities of the compounds are shown in Tables 1 and 2. Each compound was dried over phosphorus pentoxide *in vacuo* at 80°C for about 10 hr before being subjected to elementary analysis. All the melting points were determined by the capillary method, and all are given as uncorrected values.

Aoc-L-prolyl-L-proline Benzyl Ester (III). DCC (51.5 g, 0.25 mol) was stirred into a solution of Aoc-L-proline (I) (57.2 g, 0.25 mol) in methylene chloride (150 m*l*) at 0°C. Then, a solution of L-proline benzyl ester hydrochloride (II) (61 g, 0.26 mol) and triethylamine (35 m*l*, 0.25 mol) in methylene chloride (250 m*l*) was added to the reaction mixture. Stirring was continued for 1 hr at 0°C, and then the mixture was left to stand overnight at room temperature. The *N,N*-dicyclohexylurea (DCU) formed during the reaction was removed by filtration. The filtrate was concentrated under reduced pressure, and the residue was dissolved in ethyl acetate. This solution was washed successively with water, 5% sodium bicarbonate, 0.25 N hydrochloric acid, and water. The washed solution was dried over sodium sulfate and concentrated to a syrup, which was then used in the next reaction without any further purification or characterization. The homogeneity of this material was checked by thin-layer chromatography on Silica-gel G using a mixture of chloroform - methanol - acetic acid (95 : 5 : 3 v/v/v) as the solvent.[4]

Aoc-L-prolyl-L-proline (IV).[5] Hydrogen gas was bubbled through a solution of the above syrup (III) in methanol (500 m*l*) in the presence of palladium charcoal (5%, 5 g) for about 10 hr at room temperature. The extent of hydrogenolysis was checked by thin-layer chromatography on Silica-gel G using a mixture of chloroform-methanol-pyridine (95 : 5 : 3 v/v/v) as the solvent.[4] After confirmation that the reaction has been completed, the catalyst was filtered off, and the filtrate was concentrated to a syrup under reduced pressure. The residue was crystallized from a small volume of ethyl acetate in a refrigerator. The product was then collected by filtration and recrystallized from ethyl acetate and *n*-hexane; wt, 67.2 g (yield 82.5%, calcd from I).

Found: C, 58.98; H, 8.02; N, 8.72%. Calcd for $C_{16}H_{26}N_2O_5$: C, 58.88; H, 8.03; N, 8.58%.

Aoc-tri-L-proline Benzyl Ester (V). DCC (24.8 g, 0.12 mol) was stirred into a solution of IV (39.1 g, 0.12 mol) in methylene chloride (150 m*l*) at 0°C. Then, a mixture of II (30.5 g, 0.12 mol) and triethylamine (16.8 m*l*, 0.12 mol) in methylene chloride (150 m*l*) was added to the reaction mixture. Stirring was continued for 1 hr at 0°C, and then the mixture was kept overnight at room temperature. The DCU formed was removed by filtration. The solvent was replaced by ethyl acetate, and a small amount of an insoluble material was filtered off; the ethyl acetate solution was thoroughly washed in succession with water, 5% sodium bicarbonate, 0.25 N hydrochloric acid, and water. The final solution was dried over sodium sulfate and concentrated to a syrup under reduced pressure. The residue was dissolved in acetone, and a small amount of an insoluble material was removed by filtration. The solvent was evaporated off, and the residue was crystallized from ethyl acetate and *n*-hexane. Crystals were collected and recrystallized from ethyl acetate and *n*-hexane as fine needles; wt, 48 g (yield 78%).

Found: C, 65.63; H, 7.70; N, 8.17%. Calcd for $C_{28}H_{39}N_3O_6$: C, 65.47; H, 7.65; N, 8.18%.

Aoc-tri-L-proline (VI). Hydrogen was bubbled slowly through a solution of V (30.8 g, 0.06 mol) in methanol (150 m*l*) in the presence of palladium charcoal (5%, 5 g). After we had confirmed by thin-layer chromatography that the reaction was complete, the catalyst was removed by filtration; the filtrate was then concentrated under reduced pressure. The residue was dissolved in ethyl acetate with the aid of a minimum volume of methanol, and the solution was kept overnight in a refrigerator. The crude product, which separated as crystals, was collected by filtration and then recrystallized as fine needles from a mixture of ethyl acetate and a small volume of methanol; wt, 22.5 g (89.1%).

Found: C, 59.63; H, 7.88; N, 9.75%. Calcd for $C_{21}H_{33}N_3O_6$: C, 59.55; H, 7.85; N, 9.92%.

Aoc-tetra-L-proline Benzyl Ester (VII). Compounds VI (16.9 g, 0.04 mol) and II (11.0 g, 0.04 mol) were treated with DCC (8.3 g, 0.04 mol) in chloroform as has been described for the syntheses of III and V. The DCU formed was removed by filtration, the chloroform was replaced by ethyl acetate, and the solution was thoroughly washed in succession with water, 5% sodium bicarbonate, 0.25 N hydrochloric acid, and water; the washed solution was dried over sodium sulfate and then concentrated to a residue under reduced pressure. The residue was dissolved in acetone, a small amount of an insoluble material was removed by filtration, and the solution was reconcentrated. The final residue was crystallized from ethyl acetate and *n*-hexane. Crude crystals were collected by filtration and recrystallized from ethyl acetate and *n*-hexane; wt, 19.5 g (80.1%).

Found: C, 64.15; H, 7.72; N, 9.02%. Calcd for $C_{33}H_{46}N_4O_7 \cdot \frac{1}{2} H_2O$: C, 63.96; H, 7.59; N, 9.03%.

Aoc-tetra-L-proline (VIII). Compound VII (10 g, 0.016 mol) was subjected to catalytic hydrogenolysis with hydrogen and palladium charcoal in methanol, as with VI. The catalyst was removed by filtration, and the filtrate was concentrated under reduced pressure to obtain the crude product, which was crystallized from ethyl acetate and *n*-hexane. Recrystallization from the same solvent system gave the final product; wt, 7.5 g (90%).

Found: C, 59.71; H, 7.64; N, 10.75%. Calcd for $C_{26}H_{40}N_4O_7$: C, 59.98; H, 7.74; N, 10.76%.

Aoc-penta-L-proline Benzyl Ester (IX). (a) Compound V (5.3 g, 0.011 mol) was dissolved in 10 m*l* of trifluoroacetic acid, after which the mixture was allowed to react for 30 min at room temperature. The excess trifluoroacetic acid was removed by evaporation under reduced pressure, and the residual syrup was dried over sodium hydroxide *in vacuo* for one day. The dried material was then dissolved in chloroform (50 m*l*), and the solution was treated with compound IV (3.6 g, 0.011 mol) and DCC (2.3 g, 0.011 mol) in chloroform, as in the syntheses of III and V. The DCU formed was removed by filtration, and the filtrate was washed successively with 0.25 N hydrochloric acid, 5% sodium bicarbonate, and water, and dried over sodium sulfate. The dried solution was concentrated, and the residue was dissolved in hot ethyl acetate. Crystals formed when this solution was left standing in a refrigerator. The product was collected and recrystallized from ethyl acetate; wt, 5.2 g (74.5%). Found: C, 64.34; H, 7.65; N, 9.77%. Calcd for $C_{38}H_{53}N_5O_8$: C, 64.48; H, 7.55; N, 9.89%. (b) Compound VII (5.2 g, 0.01 mol) was coupled with II (2.6 g, 0.011 mol) in chloroform using DCC (2.1 g, 0.01 mol), and the reaction mixture was treated as has been described above. The product was recrystallized similarly; wt, 5.5 g (78.5%). As is shown in Table 1, the properties of this product were almost the same as those of the compound obtained above.

Found: C, 64.58; H, 7.56; N, 9.92%. Calcd for $C_{38}H_{53}N_5O_8$: C, 64.48; H, 7.55; N, 9.89%.

Aoc-penta-L-proline (X). (a) A solution of compound IX-a (3.6 g, 0.005 mol) in methanol (100 m*l*) was subjected to catalytic hydrogenolysis in the presence of palladium charcoal, as in the syntheses of IV and VI. The catalyst was filtered off, the filtrate was concentrated under reduced pressure, and the residue was recrystallized twice from a minimum volume of methanol and ethyl acetate; wt, 2.55 g (82.7%).

Found: C, 59.95; H, 7.74; N, 11.52%. Calcd for $C_{31}H_{47}N_5O_8$: C, 60.27; H, 7.67; N, 11.34%.

(b) An indistinguishable compound was also obtained from IX-b, as is shown in Table 2.

5) S. Sakakibara and N. Inukai, This Bulletin, **39**, 1567 (1966).

Aoc-hexa-L-proline Benzyl Ester (VI). An Aoc-group was removed from V (5.14 g, 0.01 mol); the triproline benzyl ester thus obtained was coupled with VI (4.24 g, 0.01 mol) in chloroform (50 m*l*) by using DCC (2.1 g, 0.01 mol), as in the synthesis of IX-a. The crude product thus obtained was recrystallized from a mixture of ethyl acetate and methanol (2 : 1 v/v); wt, 5.1 g (69.4%).

Found: C, 63.84; H, 7.54; N, 10.25%. Calcd for $C_{43}H_{60}N_6O_9$: C, 64.16; H, 7.51; N, 10.44%.

Aoc-hexa-L-proline (XII). Compound XI (4.0 g, 0.005 mol) was subjected to catalytic hydrogenolysis in methanol, as with X, VIII, VI, and IV. The catalyst was removed by filtration, and the filtrate was concentrated to a residue which was then triturated with hot ethyl acetate to remove any soluble materials. The insoluble material remaining was dissolved in methanol, and the solution was treated with active charcoal to eliminate any colored impurities. The colorless methanol solution thus obtained was concentrated to a small volume, and the product was precipitated with ethyl acetate. The precipitate was collected by filtration and recrystallized from methanol and ethyl acetate; wt, 3.0 g (84.8%).

Found: C, 60.08; H, 7.48; N, 11.70%. Calcd for $C_{36}H_{54}N_6O_9$: C, 60.48; H, 7.61; N, 11.76%.

Aoc-octa-L-proline Benzyl Ester (XIII). Compound VII (3.05 g, 0.005 mol) was treated with trifluoroacetic acid (5 m*l*) as in the case of IX-a; the tetra-L-proline benzyl ester thus obtained was coupled with VIII (2.6 g, 0.005 mol) in chloroform by using DCC (1.1 g, 0.005 mol), as has been described previously. The DCU thus formed was removed by filtration; the chloroform solution was washed successively with 0.25 N hydrochloric acid, 5% sodium bicarbonate, and water. The solution was then dried over sodium sulfate and concentrated to a residue which was subsequently purified by two precipitations from methanol and ethyl acetate; wt, 2.9 g (59.5%).

Found: C, 63.52; H, 7.50; N, 10.93%. Calcd for $C_{53}H_{74}N_8O_{11}$: C, 63.70; H, 7.47; N, 11.22%.

Aoc-octa-L-proline (XIV). Compound XIII (2.0 g, 0.002 mol) was subjected to catalytic hydrogenolysis in methanol (25 m*l*) with palladium-charcoal, as has been described for the syntheses of similar compounds. After we had confirmed that the reaction was complete, the catalyst was removed by filtration and the solution was concentrated to a residue which was subsequently from methanol and ethyl acetate; wt, 1.5 g (86%).

Found: C, 60.43; H, 7.46; N, 12.16%. Calcd for $C_{46}H_{68}N_8O_{11}$: C, 60.77; H, 7.54; N, 12.33%.