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Studies on the synthesis of Protein Analogus (Part 1) Synthesis of Polypeptides by the Method of N-carbothiophenyl-amino acids*

By

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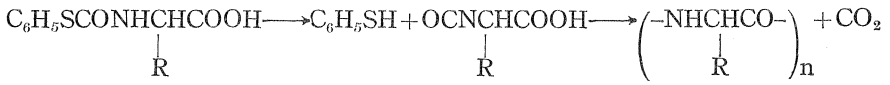
I found that N-carbothiophenyl-amino acids expell thiophenol and carbon dioxide to be polymerized in polypeptides by heating. By using this reaction, we can synthesise-polypeptides from amino acids.

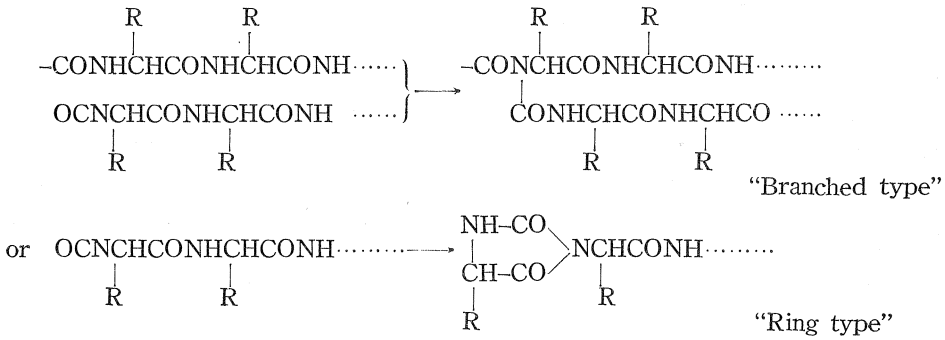
Some N-carbothiophenyl-amino acids were prepared and the heat decompositions were measured by thermobalance (Fig. 1). They begin to decompose at about 120–130 ℃ and suddenly caused decompositions at about 150–160 ℃. This decomposition reaction is considered to take the following process :

main reaction,

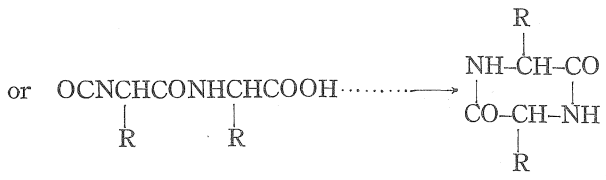


The isocyanate carboxylic acids which are intermediate, polymerise immediately to polypeptide or some parts of them react with other carbothiophenyl-amino acids and become carbothiophenyl polypeptides and then this expells thiophenol to become isocyanate polypeptides and polymerise immediately in polypeptides or combine with thiophenylamino acids or thiophenyl polypeptides and finally they polymerise in polypeptide having high molecular weight by the same process. Some subreactions are considered to take place in this reaction. Some parts of the end isocyanate group produced, react with –CO–NH– groups and form "branched type "or" ring type" and lose the polymerisability.

Subreactions,



* It was published in the Jan. of the Chem. Soc. of Japan (in Japanese), **74**, 961—965 (1953)



"Ring type"

The poly-α-amino acids which were obtained by heating over 130℃, showed low polymerisation degree, and the Nitrogen contents of them were lower than the theoretical value of $\left(\text{–NHCHCO–}\right)_n$ (R). When N–carbothiophenyl–DL–alanine was polymerized in a sealed tube by heating at 150 ℃, 180 ℃, 200 ℃ and 250 ℃ either in solid state or in toluene solution, the polymers did not contain any thiophenol, and the analytical data coresponded to the polymers of $\text{CO}\!\!-\!\!\text{NHCHCONCHCO}\left(\text{NHCHCO}\right)_n\text{OH}$ (CH_3), in which n were 3, 4, 5, 6, 7 etc.[1] These polymers were somewhat hygroscopic, showed the Biuret's reaction and were soluble in alcohol or water. At the heating of about 200 ℃, some part of diketopiperazine was produced. The melt reaction of carbobenzyloxy-glycine and carbothiophenyl–DL–alanine ethylester, carbothiophenyl–DL–phenylalanine ethylester, or carbothiophenyl-glycine ethylester, produced the compounds[2] corresponding to the following, Cbzo–$NHCH_2CONHCHCOOH$ (CH_3), $C_6H_5CH_2CH\langle\text{CO–NH}\rangle\text{CH}_2$ ($\langle\text{NH–CO}\rangle$) or Cbzo–$N(CH_2COOH)CONHCH_2COOH$.

These high temperature reactions are not suitable for the high polymerisation reaction, beause the subreaction as mentioned above occurs in such conditions.

It was found that the polypeptides having high molecular weights could be obtained without such subreactions when the monomer were heated for a long time in suitable solvent even at low temperature of 50–80 ℃, at which any decompositions were not clearly observed at solid state as shown in Fig. 1. It may be thought that the peptide bond "–CONH–" will be formed without any dissociation of isocyanate state in such conditions.

$$n\ C_6H_5SCONHCHCOOH \longrightarrow n\ C_6H_5SH + n\ CO_2 + \left(\text{–NHCHCO–}\right)_n \quad (R)$$

If the isocyanate should be liberated, it might form urethan, reacting with alcohol. Carbothiphenyl–DL–alanine ethylester did not give carboethoxy–DL–alanine ethylester and recovered itself quantitatively, when heated with alcohol at it's boiling point for a few tens of hours. As shown in Fig. 2, the heat decomposition at 130 ℃ of carbothiophenyl–DL–alanine shows at it's initial state another decomposition reaction different

1) J. Noguchi, S. Ishino and T. Hirono, The protein 3, 104 (1952) (in Japanese); A. Lindenmann, N. H. Khan, K. Hofmann, J. Am. Chem. Soc. 74, 476 (1952) obtained the hydantoinester corresponding to this formula from carbothiophenyl-dipeptide ester.

2) J. Noguchi, M. Asai, S. Ishino, the Lecuture abstracts of the 6th annual meating of the Chem. Soc. of Japan, p. 178 (1953)

from the dissociation of isocyanate, which are unobservable at 140 ℃ and 150 ℃. From these facts, I think that the solution polymerisation at low temperature of 50—80 ℃ might occur by the mechanism different from that at high temperature of over 130℃[3].

Carbothiophenyl-glycine (m. p. 155° C),[4] carbothiophenyl-DL-alanine (m. p. 138° C),[4] carbothiophenyl-β-alanine (m. p. 104 ℃), carbothiophenyl-L-leucine (syrup), carbothiophenyl-DL-phenylalanine (m. p. 56° C), carbothiophenyl-DL-α-amino lauric acid (m.p. 23 ℃), carbothiophenyl-ε-amino caproic acid (m. p. 98 ℃), carbothiophenyl-DL-alanyl-glycine (m. p. 167 ℃), carbothiophenyl-L-glutamic acid[4] and carbothiophenyl-L-glutamic acid anhydride (m.p. 167℃)[4] were prepared and these polymerisations were tested in various kinds of solvents.

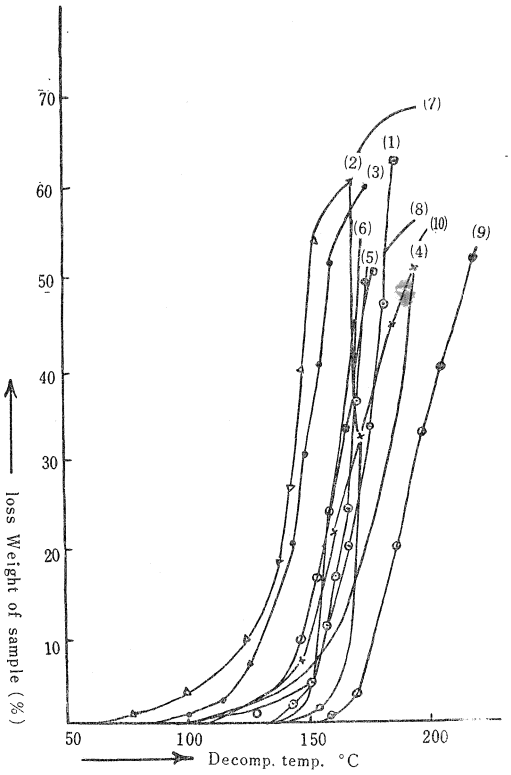


Fig. 1 Decomp. curve of each N-carbothiophenylamino-acid.

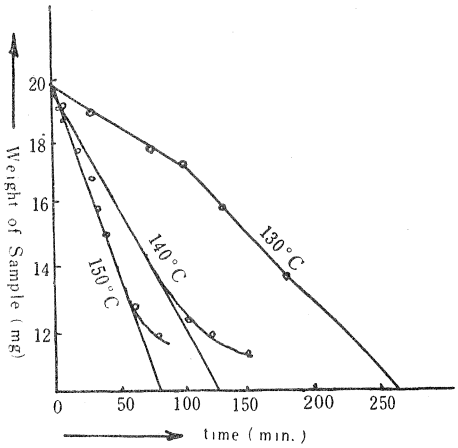


Fig. 2 Decomposition curve of N-carbothiophenyl-DL-phenylalanine·

I found that the polypeptide having a considerable high molecular weight could be prepared when the monomer was heated at 80℃ for several houndred hours in the solution of benzene or benzene containg a small quantity of pyridine. In benzene only, the velocity of polymerisation are very slow and pyridine accelerates the polymerisation. All the solution was almost completely gelatinized when the monomer was polymerized in the benzene containing 8 % pyridine (about 10 moles for the monomer) in the polymerisation of DL-phenylalanine.

This polymer was soluble in the mixed solvent of acetic acid and monochlor acetic acid (1 : 1). The viscocity by Ostwald's method and the molecular weight by osmotic

3) J. Noguchi, J. Miyamori, Biochemistry **25**, 23 (1953) (in Japanese)
4) A. Lindenmann, N. J. Khan, K. Hofmann, J. Am. Chem. Soc. **74**, 476 (1952)

method are as follows : intrinsic viscocity $[\eta]=0.068$ (g/100cc unit), molecular weight= 59,400, polymerisation degree≒450.

The polymerisability is influenced by the sorts of amino acids. L-leucine is completely gelatinized in benzene like DL-phenyl-alanine, but L-glutamic acid, ε-aminocaproic acid and DL-alanyl-glycine show lower polymerisation degrees in the same condition. But in proper conditions, dipeptides such as DL-alanyl-glycine as well as mono-amino acids can be polymerized, too.

Hitherto the only method of high polymerisation of α-amino acids was that in which the N-carboxylic acid anhydride is used,[5] but now we can also prepare the polypeptides having high molecular weights by my N-carbothiophenyl method. Moreover, ω-amino acids, such as polypeptide consisting of some kinds of amino acids, β-alanine and ε-aminocaproic acid, can be polymerized in protein order, which could not be polymerized[6] by the former method.

A detailed account of this work will be published successively.

Experimental

N-carbothiophenyl-β-alanine ethylester——β-alanine hydrochloride 15.5 g (0.12 moles) was esterified with alcohol and dry HCl. The ether solution of β-alanine ethylester was prepared by treating it with alkali. When carbothiophenyl-chloride 8.5 g was added to the cold solution, it precipitate β-alanine ester hydrochloride after a short time. It was filtered and the ether solution was washed several times with water containing a little hydrochloric acid. When it was dried with Na_2SO_4, concentrated to dryness, it turned into white plate crystal. It was recrystalized with ether and petrol-ether. Yield 10.2 g, m. p. 52 ℃. It is easily soluble in alcohol, ether and ethyl acetate, and insoluble in water.

Analysis found N=5.33%, S=12.14%

for $C_{12}H_{15}O_3NS$ calc. N=5.53%, S=12.65%

N-carbothiophenyl-β-alanine——N-carbothiophenyl-β-alanine ethylester 9.2 g was dissolved in the mixture of acetic acid 20 cc and conc. HCl 20 cc. It was heated under flame for ten minutes, cooled immediately after that, and then had water 40 cc added to it and was left at room temperature. Being lustrous crystal, it was washed with diluted HCl and water, and dried in vaccuum. Yield 6.3 g (77 % of the theoretical). m. p. 104 ℃.

Analysis found N=5.81%, S=13.99%

for $C_{10}H_{11}O_3NS$ calc. N=6.22%, S=14.22%

N-carbothiophenyl-L-leucine ethylester——The ether solution of L-leucine ethylester which was prepared from L-leucine ethylester HCl 50 g was cooled and had carbothiophenylchloride 21 g added to it drop by drop under stirring and cooling. The

5) R. B. Woodward, C. H. Shramm, J. Am. Chem. Soc. **69**, 1551 (1947)

6) J. Noguchi, The chemistry of highpolymer, **6**, 200 (1949) (in Japanese).

produced precipitate was filtered and after 2–3 hours the ether solution washed with HCl-acidified water several times, dried with Na_2SO_4, and concentrated. It was syrup and did not crystalize. Yield 39.0 g (almost quantitatively)

N analysis found N=4.70%

for $C_{15}H_{21}O_3NS$ calc. N=4.75%

N-carbothiophenyl-L-leucine——The mixture of acetic acid 100 cc and conc. HCl 50 cc was added to N-carbothiophenyl-L-leucine ethylester 28.0 g, boiled under flame for 25 minutes and then cooled. It was concentrated under reduced pressure below 45° C, dissolved in ether 50 cc, washed with HCl-acidified water, dried with Na_2SO_4 and evaporated ether. The residue was clear syrup. It was not crystalized by several solvents.

N analysis found N=5.19%

for $C_{13}H_{17}O_3NS$ calc. N=5.25%

N-carbothiophenyl-DL-*phenylalanine ethylester*——The ether solution of DL-phenylalanine ethylester which was prepared from DL-phenylalanine ethylester HCl 23 g was cooled and carbothiophenylchloride 16 g was slowly added to it below 0°C. After overnight, the precipitate was filtered off and the ether solution was washed with HCl-acidified water, dried with Na_2SO_4 and evaporated ether. It became crystal and was washed with ligroin and dried. Yield 13.0 g (43% of the theoretical). m. p.63—65°C.

N analysis found N=3.99%

for $C_{18}H_{19}O_3NS$ calc. N=4.26%

N-*carbothiophenyl*-DL-*phenylalanine*——N-carbothiophenyl-DL-phenylalanine ethylester 8 g was dissolved in the mixture of acetic acid 20 cc and conc. HCl 15 cc, boiled for 10 minuts under refluxing and cooled immediately after that. When it was diluted with water 35 cc, oily matter separated. It was extracted with ether and the ether extract was washed with HCl-acidified water, dried with Na_2SO_4 and concentrated beolw 35 °C. A part of it was taken, dissolved in ether, rubbed with petrol-ether and left in a cold state. It became crystal. Using this crystal as (so-called) 'seed', the main part of the syrup was rubbed with petrol-ether and kept for a week in an ice box. It became crystal. Yield 6.3 g (86% of the theoretical). m. p. 56° C.

N analysis found N=4.61%

for $C_{16}H_{15}O_3NS$ calc. N=4.66%

N-carbothiophenyl-DL-α-aminolauric acid——The ether solution of α-aminolauric ester which was prepared from α-aminolaulic acid ethylester HCl 10 g (m. p. 75 °C) was cooled below 0 °C and the ether solution of carbothiophenylchloride 4 g was slowly added to it. The produced α-aminolauric ester HCl was filtered and the solution washed with HCl-acidified water, dried with Na_2SO_4 and ether was evaporated. It was syrup and did not crystalize. It was dissolved in the mixture of acetic acid 10 cc and conc. HCl 5 cc, boiled for 30 minuts and after cooling, it was diluted with water. Oily precipitate was obtained, washed with water and kept in an ice box. When it was

rubbed, it became crystal. It was washed with water and dried. It was white crystal. Yield 5.5 g (88% of the theoretical). m. p. 23 °C.

N analysis found N=4.47%

for $C_{16}H_{29}O_3NS$ calc. N=3.69%

N-carbothiophenyl-ε-aminocaproic acid——The ether solution of ε-aminocaproic ester which was prepared from ε-aminocaproic acid ethylester HCl 50 g was cooled, and the ether solution of carbothiophenylchloride 20 g was slowly added to it. It was left for 4 hours at room temperature, the precipitate was filtered and the ether solution was washed with water several times and dried with Na_2SO_4. When ether was evaporated, it became syrup and did not crystalize. Yield 20 g. The syrup was dissolved in the mixture of acetic acid 25 cc and conc. HCl 25 cc, boiled for 10 minuts, left for 2 hours at room temperature and diluted with water 50 cc. It became crystal, filtered off, washed with HCl-acidified water and dried. Crude 18.5 g (almost quantitatively). It was recrystalized with ether. Yield 11 g (61% of the theoretical). m. p. 98° C.

N analysis found N=5.21%

for $C_{13}H_{17}O_3NS$ calc. N=5.24%

N-carbothiophenyl-DL-alanyl-glycine ethylester——The mixture of $CHCl_3$ 20 cc and $SOCl_2$ 4.5 g was added to N-carbothiophenyl-DL-alanine 7 g and warmed at about 40 °C. After the vesication ceased and the solution became clear, it was concentrated again. The residue was dissolved in $CHCl_3$ 25 cc. The ether solution containing glycine free ester 6 g was cooled at −7——10 °C and the chloroform solution of acidchloride was added to it under stirring. After the addition was finished, it was left for about 30 minuts at the same temperature and then at room temperature for overnight. The produced crystal was filtered, washed with water and dried. The filtrate was washed with acidified water, dried with Na_2SO_4 and evaporated to dryness. The combined crystal was 7.2 g. It was recrystalized with the same volume of benzene. m. p. 119—120 °C.

N analysis found N=8.95%

for $C_{14}H_{18}O_4N_2S$ calc. N=9.04%

N-carbothiophenyl-DL-alanyl-glycine——N-carbothiophenyl-DL-alanylglycine ethylester 7.2 g was suspended to the mixture of 8 N-HCl 20 cc and acetone 40 cc, heated at boiling point of water for 30 minuts and cooled immediately after that. It was concentrated below 35 °C. White crystal was obtained, washed with acidified water and dried. Yield 4.8 g m. p. 167—168 °C.

N analysis found N=9.92%

for $C_{12}H_{14}O_4N_2S$ calc. N=9.94%